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October 11, 2000

Attorney Docket No.: 10448-088001

Box Patent Application
Commissioner for Patents
Washington, DC 20231

Presented for filing is a new original patent application of:

Applicant: NADINE WEICH

Title: 8843, A NOVEL HUMAN DUAL SPECIFICITY PHOSPHATASE AND USES THEREOF

Enclosed are the following papers, including those required to receive a filing date under 37 CFR §1.53(b):

	Pages
Specification	98
Claims	8
Abstract	1
Declaration	[Unexecuted]
Drawing(s)	8

Enclosures:

— Nucleotide and/or amino acid sequence listing including:
Computer readable copy.
Paper copy, 6 pages.
Verification statements, 2 pages.

— Postcard.

Basic filing fee	$710
Total claims in excess of 20 times $18	$216
Independent claims in excess of 3 times $80	$640

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Fee for multiple dependent claims	$0
Total filing fee:	$1566

A check for the filing fee is enclosed. Please apply any other required fees or any credits to deposit account 06-1050, referencing the attorney docket number shown above.

If this application is found to be incomplete, or if a telephone conference would otherwise be helpful, please call the undersigned at (617) 542-5070.

Kindly acknowledge receipt of this application by returning the enclosed postcard.

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Attorney's Docket No.: 10448-088001 / MPI2000-345; 100-645590

APPLICATION

FOR

UNITED STATES LETTERS PATENT

TITLE: 8843, A NOVEL HUMAN DUAL SPECIFICITY PHOSPHATASE FAMILY MEMBER AND USES THEREOF

APPLICANT: NADINE WEICH

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PATENT
ATTORNEY DOCKET NO.: 10448-088001

JC926 U.S. PTO 09/686673 10/11/00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Nadine Weich
Serial No. :
Filed : Herewith
Title : 8843, A NOVEL HUMAN DUAL SPECIFICITY PHOSPHATASE FAMILY MEMBER AND USES THEREOF

Art Unit :
Examiner :

Commissioner for Patents
Washington, D.C. 20231

VERIFIED STATEMENT UNDER 37 CFR §1.821(f)

I, Jennifer H. Payne, declare that I personally prepared the paper and the computer-readable copy of the Sequence Listing filed herewith for the above-identified application and that the content of both is the same.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of The United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 9-21-00

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8843, A NOVEL HUMAN DUAL SPECIFICITY PHOSPHATASE FAMILY MEMBER AND USES THEREOF

Background of the Invention

The intracellular phosphorylation of proteins is critical for a plethora of regulatory and signalling mechanisms in eukaryotic cells. Phosphorylation events can govern a wide range of cellular processes, including cell proliferation, differentiation, transcription, and morphology. Serine/threonine protein kinases, also called serine protein kinases, are frequently utilized in signalling cascades as the activity of these enzymes can be finely regulated by stimuli. A common stimulus is phosphorylation of the serine protein kinase itself. Hence, signalling pathways, such as the MAP protein kinase cascade, can contain multiple proteins kinases which are sequentially activated. Ultimately, kinase cascades can result in the phosphorylation of cytoskeletal proteins, transcription factors, and biosynthetic enzymes. Another class of kinases includes the receptor tyrosine kinases. Activated receptor tyrosine kinases not only autophosphorylate, but phosphorylate other intracellular signalling molecules, including those specifically bound to autophosphorylated receptors.

An essential component of the aforementioned signalling pathways is the ability of the cell to desensitize, recycle, and counteract phosphorylation signals. The cell primarily utilizes enzymes, termed phosphatases, which remove the phosphate on tyrosine, serine, and threonine side chains. Dual specificity phosphatases hydrolyze phosphotyrosine, phosphothreonine, and phosphoserine residues (for a review, see, e.g., Fauman and Saper (1996) *Trends in Biochem.* 21:412). This class of proteins is exemplified by the VH1 or vaccinia virus late H1 gene protein, whose catalytic activity is required for vaccinia virus replication. A human homolog of VH1, VHR, has also been identified. VH1-like dual specificity phosphatase can also include the phosphatases PAC-1 and CL100/MKP-1, hVH-2/MKP-2, hVH-3, MKP-3, MKP-X, MKP-4, hVH-5, and M3/6 proteins. The PAC-1 and CL100 proteins hydrolyze phosphothreonine and phosphotyrosine residues on phosphorylated MAP (mitogen activated protein) kinases. In order to modulate signalling events, the activity and expression of dual specificity phosphatases can be finely regulated. For example, the PAC-1 and CL100 phosphatase can be induced by growth factors (Keyse, S (1995) *Biochim. Biophys. Acta*1265:152-160).

Thus, the function of dual specificity phosphatase proteins can be critical for the regulation of cellular processes such as proliferation and differentiation.

Summary of the Invention

The present invention is based, in part, on the discovery of a novel dual specificity phosphatase family member, referred to herein as "8843". The nucleotide sequence of a cDNA encoding 8843 is shown in SEQ ID NO:1, and the amino acid sequence of an 8843 polypeptide is shown in SEQ ID NO:2. In addition, the nucleotide sequences of the coding region are depicted in SEQ ID NO:3.

Accordingly, in one aspect, the invention features a nucleic acid molecule which encodes an 8843 protein or polypeptide, e.g., a biologically active portion of the 8843 protein. In a preferred embodiment the isolated nucleic acid molecule encodes a polypeptide having the amino acid sequence of SEQ ID NO:2. In other embodiments, the invention provides isolated 8843 nucleic acid molecules having the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number ____. In still other embodiments, the invention provides nucleic acid molecules that are substantially identical (e.g., naturally occurring allelic variants) to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number ____. In other embodiments, the invention provides a nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number ____, wherein the nucleic acid encodes a full length 8843 protein or an active fragment thereof.

In a related aspect, the invention further provides nucleic acid constructs which include an 8843 nucleic acid molecule described herein. In certain embodiments, the nucleic acid molecules of the invention are operatively linked to native or heterologous regulatory sequences. Also included, are vectors and host cells containing the 8843 nucleic acid molecules of the invention e.g., vectors and host cells suitable for producing 8843 nucleic acid molecules and polypeptides.

In another related aspect, the invention provides nucleic acid fragments suitable as primers or hybridization probes for the detection of 8843-encoding nucleic acids.

In still another related aspect, isolated nucleic acid molecules that are antisense to an 8843 encoding nucleic acid molecule are provided.

In another aspect, the invention features, 8843 polypeptides, and biologically active or antigenic fragments thereof that are useful, e.g., as reagents or targets in assays applicable

to treatment and diagnosis of 8843-mediated or -related disorders, e.g., erythroid associated disorders. In another embodiment, the invention provides 8843 polypeptides having an 8843 activity. Preferred polypeptides are 8843 proteins including at least one dual specificity phosphatase domain, and, preferably, having an 8843 activity, e.g., an 8843 activity as described herein.

In other embodiments, the invention provides 8843 polypeptides, e.g., an 8843 polypeptide having the amino acid sequence shown in SEQ ID NO:2; the amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC Accession Number ___; an amino acid sequence that is substantially identical to the amino acid sequence shown in SEQ ID NO:2; or an amino acid sequence encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number ____, wherein the nucleic acid encodes a full length 8843 protein or an active fragment thereof.

In a related aspect, the invention further provides nucleic acid constructs which include an 8843 nucleic acid molecule described herein.

In a related aspect, the invention provides 8843 polypeptides or fragments operatively linked to non- 8843 polypeptides to form fusion proteins.

In another aspect, the invention features antibodies and antigen-binding fragments thereof, that react with, or more preferably specifically bind 8843 polypeptides.

In another aspect, the invention provides methods of screening for compounds that modulate the expression or activity of the 8843 polypeptides or nucleic acids.

In still another aspect, the invention provides a process for modulating 8843 polypeptide or nucleic acid expression or activity, e.g. using the screened compounds. In certain embodiments, the methods involve treatment of conditions related to aberrant activity or expression of the 8843 polypeptides or nucleic acids, such as conditions involving aberrant (e.g., enhanced or deficient) erythroid cell activity (e.g., aberrant erythroblast proliferation, e.g., an erythroleukemia, and/or aberrant erythroblast differentiation, e.g., an anemia).

The invention also provides assays for determining the activity of or the presence or absence of 8843 polypeptides or nucleic acid molecules in a biological sample, including for disease diagnosis.

In further aspect the invention provides assays for determining the presence or absence of a genetic alteration in an 8843 polypeptide or nucleic acid molecule, including for disease diagnosis.

In another aspect, the invention features a method for identifying a compound which interacts with, e.g., binds to, or modulates an activity of an 8834 polypeptide or nucleic acid. The method includes contacting the 8834 polypeptide or nucleic acid, or a cell containing, e.g., expressing, the 8834 polypeptide or nucleic acid with a test compound, and determining whether the polypeptide or nucleic acid interacts with, e.g., binds to, the test compound, or whether the test compound modulates an activity of an 8834 polypeptide.

In a preferred embodiment, the interaction, e.g., binding, of the test compound to the 8834 polypeptide or nucleic acid is detected by one of the following methods: a) detection of binding (e.g., direct binding) by labeling the test compound or the 8843 polypeptide or nucleic acid with a detectable signal, e.g., a radioactive metal ion or a fluorescent signal, and detecting the bound labeled complex of the test compound/polypeptide or nucleic acid binding relative to the unbound fraction; b) detection of binding using a competition binding assay; or c) detection of binding using an assay for 8843 polypeptide activity, e.g., protein phosphatase activity.

In a preferred embodiment, the 8834 activity is hematopoiesis, e.g., erythropoiesis.

In a preferred embodiment, the 8834 polypeptide has an amino acid sequence identical to, or substantially identical to, SEQ ID NO:2. In other embodiments, the 8834 polypeptide is a fragment of at least 15, 20, 50, 100, 150, 180, or more contiguous amino acids of SEQ ID NO:2.

In a preferred embodiment, the 8834 nucleic acid has a nucleotide sequence identical to, or substantially identical to, SEQ ID NO:1 or 3. In other embodiments, the 8834 nucleic acid is a fragment of at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, or more contiguous nucleotides of SEQ ID NO:1 or 3.

In preferred embodiments, the compound is a peptide, a phosphopeptide, a small molecule, e.g., a member of a combinatorial library, or an antibody. In additional preferred embodiments, the compound is an antisense, a ribozyme, or a triple helix molecule.

In another aspect, the invention features a method of identifying a compound which modulates the activity (e.g., hematopoiesis, e.g., erythropoiesis) or expression of an 8834 polypeptide or nucleic acid. The method includes contacting the polypeptide or nucleic acid

with a test compound; and determining the effect of the test compound on the activity or expression of the polypeptide or nucleic acid.

In a preferred embodiment, the 8834 polypeptide has an amino acid sequence identical to, or substantially identical to, SEQ ID NO:2. In other embodiments, the 8834 polypeptide is a fragment of at least 15, 20, 50, 100, 150, 180, or more contiguous amino acids of SEQ ID NO:2.

In a preferred embodiment, the determined activity is protein phosphatase activity or erythropoiesis.

In a preferred embodiment, the 8834 nucleic acid has a nucleotide sequence identical to, or substantially identical to, SEQ ID NO:1 or 3. In other embodiments, the 8834 nucleic acid is a fragment of at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, or more contiguous nucleotides of SEQ ID NO:1 or 3.

In a preferred embodiment, the agent modulates (e.g., increases or decreases) expression of the 8843 nucleic acid by, e.g., modulating transcription, mRNA stability, etc.

In preferred embodiments, the agent is a peptide, a phosphopeptide, a small molecule, e.g., a member of a combinatorial library, or an antibody. In additional preferred embodiments, the agent is an antisense, a ribozyme, or a triple helix molecule.

In a preferred embodiment, the contacting step occurs *in vitro* or *ex vivo*.

In a preferred embodiment, the contacting step occurs *in vivo*. For example, the contacting step can occur in an animal model for an erythroid-associated disorder.

In yet another aspect, the invention features a method for modulating (e.g., increasing or decreasing) the activity or expression of an 8834 polypeptide or nucleic acid, comprising contacting the 8843 polypeptide or nucleic acid, or a cell containing (e.g., expressing) the 8843 polypeptide or nucleic acid with a agent which interacts with, e.g., binds to, the 8843 polypeptide or nucleic acid in a sufficient concentration to modulate the activity or expression of the polypeptide or nucleic acid.

In a preferred embodiment, the 8834 polypeptide has an amino acid sequence identical to, or substantially identical to, SEQ ID NO:2. In other embodiments, the 8834 polypeptide is a fragment of at least 15, 20, 50, 100, 150, 180, or more contiguous amino acids of SEQ ID NO:2.

In a preferred embodiment, the determined activity is protein phosphatase activity.

In a preferred embodiment, the 8834 nucleic acid has a nucleotide sequence identical to, or substantially identical to, SEQ ID NO:1 or 3. In other embodiments, the 8834 nucleic

acid is a fragment of at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, or more contiguous nucleotides of SEQ ID NO:1 or 3.

In a preferred embodiment, the agent modulates (e.g., increases or decreases) expression of the 8843 nucleic acid by, e.g., modulating transcription, nucleic acid stability, etc.

In preferred embodiments, the agent is a peptide, a phosphopeptide, a small molecule, e.g., a member of a combinatorial library, or an antibody, or any combination thereof. In additional preferred embodiments, the agent is an antisense, a ribozyme, or a triple helix molecule, or an 8843 nucleic acid, or any combination thereof.

In still another aspect, the invention features a method of modulating (e.g., enhancing or inhibiting) the proliferation, survival, or differentiation of a cell, e.g., an 8834-expressing cell, e.g., a hematopoietic cell (e.g., a bone marrow cell, such as a CD34 positive cell or a cell expressing a glycophorin A protein). The method includes contacting the cell with a agent that modulates the activity or expression of an 8834 polypeptide or nucleic acid, in an amount effective to modulate the proliferation and/or differentiation of the cell.

In a preferred embodiment, the 8834 polypeptide has an amino acid sequence identical to, or substantially identical to, SEQ ID NO:2. In other embodiments, the 8834 polypeptide is a fragment of at least 15, 20, 50, 100, 150, 180, or more contiguous amino acids of SEQ ID NO:2.

In a preferred embodiment, the 8834 nucleic acid has a nucleotide sequence identical to, or substantially identical to, SEQ ID NO:1 or 3. In other embodiments, the 8834 nucleic acid is a fragment of at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, or more contiguous nucleotides of SEQ ID NO:1 or 3.

In a preferred embodiment, the agent modulates (e.g., increases or decreases) protein phosphatase activity.

In a preferred embodiment, the agent modulates (e.g., increases or decreases) expression of the 8843 nucleic acid by, e.g., modulating transcription, mRNA stability, etc.

In preferred embodiments, the agent is a peptide, a phosphopeptide, a small molecule, e.g., a member of a combinatorial library, or an antibody. The antibody can be conjugated to a therapeutic moiety selected from the group consisting of a cytotoxin, a cytotoxic agent and a radioactive metal ion.

In additional preferred embodiments, the agent is an antisense, a ribozyme, or a triple helix molecule, or an 8843 nucleic acid, or any combination thereof.

In a preferred embodiment, the agent is administered in combination with a cytotoxic agent.

In a preferred embodiment, the cell, e.g., the 8834-expressing cell, is a pluripotent hematopoietic stem cell, e.g., a granulocytic, monocytic, erythroid, megakaryocytic, and lymphoid cell, or a precursor cell thereof. Examples of such cells include myelocytic cells (polymorphoneuclear cells), erythrocytic cells, lymphocytes, monocytes, reticular cells, plasma cells and megakaryocytes, as well as stem cells for the different lineages, and precursors for the committed progenitor cells, for example, precursors of red blood cells (erythroblasts), macrophages (monoblasts), platelets (megakaryocytes), polymorphoneuclear leucocytes (myeloblasts), and lymphocytes (lymphoblasts).

In a preferred embodiment, the cell, e.g., the 8834-expressing cell, is a bone marrow cell, e.g., a CD34-expressing cell. Examples of CD34-expressing cells include immature haematopoietic precursor cells, haematopoietic colony-forming cells in bone marrow, including unipotent (CFU-GM, BFU-E) and pluripotent progenitors (CFU-GEMM, CFU-Mix and CFU-blast); as well as stromal cell precursors, terminal deoxynucleotidyl transferase (TdT) expressing B- and T-lymphoid precursors, early myeloid cells and early erythroid cells.

In another preferred embodiment, the cell is a progenitor cell, e.g., a CFU-GEMM (colony forming unit – granulocyte, erythrocyte, macrophage, megakaryocyte), a CFU-E (colony forming unit – erythrocyte), a CFU-GM (colony forming unit – granulocyte, monocytes), or a CFU-G (colony forming unit – granulocyte).

In one embodiment, the cell is a neutrophil precursor, e.g., a CFU-GM, CFU-G (colony forming unit – granulocyte), myeloblast, promyelocyte, myelocyte, a metamyelocyte, or a band cell. In another embodiment, the cell is platelet precursor, e.g., a promegakaryoblast, a megakaryoblast, or a megakaryocyte. In still another embodiment, the cell is a monoblast or other macrophage precursor, a lymphoblast.

In a preferred embodiment, the cell is a BFU-E (blast forming unit – erythroid), CFU-E, normoblast or erythroblast, a basophilic pronormoblast, polychromatophilic pronormoblast, orthochromatophilic pronormoblast, a reticulocyte, or erythrocyte.

In a preferred embodiment, the cell, e.g., the 8834-expressing cell, is an erythroid cell, e.g., an erythroid progenitor or differentiated cell, e.g., an erythrocyte, or a cell expressing a glycophorin A protein.

In a preferred embodiment, the cell, e.g., the 8834-expressing cell, is further contacted with a protein selected from the group consisting of G-CSF, GM-CSF, stem cell factor, Flt-3 ligand, IL-3, IL-4, thrombopoietin, and erythropoietin. Preferably, the protein is erythropoietin. The protein contacting step can occur before, at the same time, or after the agent is contacted. The protein contacting step can be effected *in vitro* or *ex vivo*. For example, the cell, e.g., the 8834-expressing cell is obtained from a subject, e.g., a patient, and contacted with the protein *ex vivo*. The treated cell can be re-introduced into the subject. Alternatively, the protein contacting step can occur *in vivo*.

In a preferred embodiment, the agent and the 8834-polypeptide or nucleic acid are contacted *in vitro* or *ex vivo*.

In a preferred embodiment, the contacting step is effected *in vivo* in a subject, e.g., as part of a therapeutic or prophylactic protocol. Preferably, the subject is a human, e.g., a patient with an erythroid-associated disorder. For example, the subject can be a patient with an anemia, e.g., a drug- (e.g., chemotherapy-) induced anemia, hemolytic anemia, aberrant erythropoiesis, secondary anemia in nonhematolic disorders, anemia of chronic disease such as chronic renal failure; endocrine deficiency disease; and/or erythrocytosis (e.g., polycythemia). Preferably, the anemia is a drug- (e.g., chemotherapy-) induced anemia. Alternatively, the subject can be a cancer patient, e.g., a patient with leukemic cancer, e.g., an erythroid leukemia, or a carcinoma, e.g., a renal carcinoma.

The contacting step(s) can be repeated.

In a preferred embodiment, the agent decreases the proliferation and/or enhances the differentiation of the cell, e.g., the 8834-expressing cell, e.g., erythroid cell. Such agents can be used to treat or prevent cancers, e.g., leukemic cancers such as erythroid leukemias, or carcinomas, e.g., renal carcinomas.

In a preferred embodiment, the agent increases the number of erythroid cells, by e.g., increasing the proliferation, survival, and/or stimulating the differentiation, of eythroid progenitor cells. Such agents can be used to treat or prevent anemias, e.g., a drug- (e.g., chemotherapy-) induced anemia, hemolytic anemias, aberrant erythropoiesis, secondary anemias in nonhematolic disorders, anemias of chronic diseases such as chronic renal failure; endocrine deficiency diseases; and/or erythrocytosis (e.g., polycythemias). Preferably, the anemia is a drug- (e.g., chemotherapy-) induced anemia.

In a preferred embodiment, the agent increases the number of erythroid cells, by e.g., increasing the proliferation, survival, and/or stimulating the differentiation, of granulocytic

and monocytic progenitor cells, e.g., CFU-GM, CFU-G (colony forming unit – granulocyte), myeloblast, promyelocyte, myelocyte, a metamyelocyte, or a band cell. Such agents can be used to treat or prevent neutroponeia and granulocytopenia, e.g., conditions caused by cytotoxic chemotherapy, AIDS, congenital and cyclic neutropenia, myelodysplastic syndromes, or aplastic anemia.

In another aspect, the invention features a method of modulating hematopoiesis, e.g., erythropoiesis, comprising contacting a hematopoietic cell, e.g., an erythroid cell (e.g., an 8834-expressing cell), with a agent that increases or decreases the activity or expression of an 8834 polypeptide or nucleic acid, thereby modulating the differentiation of the hematopoietic cell, e.g., erythroid cell.

In a preferred embodiment, the 8834 polypeptide has an amino acid sequence identical to, or substantially identical to, SEQ ID NO:2. In other embodiments, the 8834 polypeptide is a fragment of at least 15, 20, 50, 100, 150, 180, or more contiguous amino acids of SEQ ID NO:2.

In a preferred embodiment, the 8834 nucleic acid has a nucleotide sequence identical to, or substantially identical to, SEQ ID NO:1 or 3. In other embodiments, the 8834 nucleic acid is a fragment of at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, or more contiguous nucleotides of SEQ ID NO:1 or 3.

In a preferred embodiment, the agent modulates (e.g., increases or decreases) protein phosphatase activity.

In preferred embodiments, the agent is a peptide, a phosphopeptide, a small molecule, e.g., a member of a combinatorial library, or an antibody. The antibody can be conjugated to a therapeutic moiety selected from the group consisting of a cytotoxin, a cytotoxic agent and a radioactive metal ion.

In additional preferred embodiments, the agent is an antisense, a ribozyme, or a triple helix molecule, or an 8843 nucleic acid, or any combination thereof.

In a preferred embodiment, the agent is administered in combination with a cytotoxic agent.

In a preferred embodiment, the hematopoietic cell is is a pluripotent hematopoietic stem cell, e.g., a cell that can give rise to progenitors of the granulocytic, monocytic, erythroid, megakaryocytic, and lymphoid lineages.

In a preferred embodiment, the erythroid cell is an erythroid progenitor or differentiated cell, e.g., an erythrocyte, or a cell expressing a glycophorin A protein.

In a preferred embodiment, the agent and the 8834-polypeptide or nucleic acid are contacted *in vitro* or *ex vivo*.

In a preferred embodiment, the contacting step is effected *in vivo* in a subject, e.g., as part of a therapeutic or prophylactic protocol. Preferably, the subject is a human, e.g., a patient with an erythroid-associated disorder. For example, the subject can be a patient with an anemia, e.g., hemolytic anemia, aberrant erythropoiesis, secondary anemia in nonhematolic disorders, anemia of chronic disease such as chronic renal failure; endocrine deficiency disease; and/or erythrocytosis (e.g., polycythemia). Alternatively, the subject can be a cancer patient, e.g., a patient with leukemic cancer, e.g., an erythroid leukemia.

In a preferred embodiment, the method further includes contacting of the erythroid cell with a protein selected from the group consisting of G-CSF, GM-CSF, stem cell factor, Flt-3 ligand, IL-3, IL-4, thrombopoietin, and erythropoietin. Preferably, the protein is erythropoietin. The protein contacting step can occur before, at the same time, or after the agent is contacted. The protein contacting step can be effected *in vitro* or *ex vivo*. For example, the cell, e.g., the erythroid cell can be obtained from a subject, e.g., a patient, and contacted with the protein *ex vivo*. The treated cell can be re-introduced into the subject. Alternatively, the protein contacting step can occur *in vivo*.

The contacting step(s) can be repeated.

In a preferred embodiment, the agent increases the number of erythroid cells, by e.g., increasing the proliferation, survival, and/or stimulating the differentiation, of eythroid progenitor cells, in the subject. Such agents can be used to treat anemias, e.g., hemolytic anemias, aberrant erythropoiesis, secondary anemias in nonhematolic disorders, anemias of chronic diseases such as chronic renal failure; endocrine deficiency diseases; and/or erythrocytosis (e.g., polycythemias).

In yet another aspect, the invention features a method of treating or preventing a hematopoietic disorder, e.g., an erythroid-associated disorder, in a subject. The method includes administering to the subject an effective amount of a agent that modulates the activity or expression of an 8834 polypeptide or nucleic acid such that the hematopoietic disorder is ameliorated or prevented.

In a preferred embodiment, the 8834 polypeptide has an amino acid sequence identical to, or substantially identical to, SEQ ID NO:2. In other embodiments, the 8834 polypeptide is a fragment of at least 15, 20, 50, 100, 150, 180, or more contiguous amino acids of SEQ ID NO:2.

In a preferred embodiment, the 8834 nucleic acid has a nucleotide sequence identical to, or substantially identical to, SEQ ID NO:1 or 3. In other embodiments, the 8834 nucleic acid is a fragment of at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, or more contiguous nucleotides of SEQ ID NO:1 or 3.

In a preferred embodiment, the agent modulates (e.g., increases or decreases) protein phosphatase activity.

In a preferred embodiment, the agent modulates (e.g., increases or decreases) expression of the 8843 nucleic acid by, e.g., modulating transcription, mRNA stability, etc.

In preferred embodiments, the agent is a peptide, a phosphopeptide, a small molecule, e.g., a member of a combinatorial library, or an antibody, or any combination thereof. The antibody can be conjugated to a therapeutic moiety selected from the group consisting of a cytotoxin, a cytotoxic agent and a radioactive metal ion.

In additional preferred embodiments, the agent is an antisense, a ribozyme, or a triple helix molecule, or an 8843 nucleic acid, or any combination thereof.

In a preferred embodiment, the agent is administered in combination with a cytotoxic agent.

In a preferred embodiment, the subject is a human, e.g., a patient with a hematopoietic disorder, e.g., an erythroid-associated disorder. For example, the subject can be a patient with an anemia, e.g., a drug- (e.g., chemotherapy-) induced anemia, hemolytic anemia, aberrant erythropoiesis, secondary anemia in nonhematolic disorders, anemia of chronic disease such as chronic renal failure; endocrine deficiency disease; and/or erythrocytosis (e.g., polycythemia). Preferably, the anemia is a drug- (e.g., chemotherapy-) induced anemia. Alternatively, the subject can be a cancer patient, e.g., a patient with leukemic cancer, e.g., an erythroid leukemia, or a patient with a carcinoma, e.g., a renal carcinoma.

In a preferred embodiment, the agent decreases the proliferation and/or enhances the differentiation of a cell, e.g., an 8834-expressing cell, e.g., an erythroid cell, in the subject. Such agents can be used to treat or prevent cancers, e.g., leukemic cancers such as erythroid leukemias, or carcinomas, e.g., renal carcinomas.

In a preferred embodiment, the agent increases the number of erythroid cells, by e.g., increasing the proliferation, and/or stimulating the differentiation, of eythroid progenitor cells, in the subject. Such agents can be used to treat anemias, e.g., a drug- (e.g., chemotherapy-) induced anemia, hemolytic anemias, aberrant erythropoiesis, secondary

anemias in nonhematolic disorders, anemias of chronic diseases such as chronic renal failure; endocrine deficiency diseases; and/or erythrocytosis (e.g., polycythemias).

In a preferred embodiment, the hematopoietic disorder is a differentiative disorder, e.g., a disorder of granulocyte, monocytes or macrophage, megakaryocyte, or lymphocyte differentiation. For example, the disorder is a disorder of granulopoiesis, lymphopiesis, or megakaryopoiesis. In another embodiment, the hematopoietic disorder is a proliferative disorder, e.g., a disorder of granulocyte, monocytes or marcophage, megakaryocyte, or lymphocyte progenitor cell proliferation.

In a preferred embodiment, the disorder is an erythroid-associated disorder. Examples of erythroid-associated disorder include a drug- (e.g., chemotherapy-) induced anemia, hemolytic anemia, aberrant erythropoiesis, secondary anemia in nonhematolic disorders, anemia of chronic disease such as chronic renal failure; endocrine deficiency disease; and/or erythrocytosis (e.g., polycythemia).

In a preferred embodiment, the disorder is a cancer, e.g., a leukemic cancer, e.g., an erythroid leukemia, or a carcinoma, e.g., a renal carcinoma.

In a preferred embodiment, the method further includes administering an effective amount of a protein selected from the group consisting of G-CSF, GM-CSF, stem cell factor, Flt-3 ligand, IL-3, IL-4, thrombopoietin, and erythropoietin to the subject. Preferably, the protein is erythropoietin. The protein can be administered before, at the same time or after, administration of the agent.

The administration of the agent and/or protein can be repeated.

In still another aspect, the invention features a method for evaluating the efficacy of a treatment of a disorder, in a subject. The method includes treating a subject with a protocol under evaluation; assessing the expression of an 8834 nucleic acid or 8834 polypeptide, such that a change in the level of 8834 nucleic acid or 8834 polypeptide after treatment, relative to the level before treatment, is indicative of the efficacy of the treatment of the disorder.

In a preferred embodiment, the disorder is an erythroid-associated disorder. Examples of erythroid-associated disorders include anemias, e.g., a drug- (e.g., chemotherapy-) induced anemia, hemolytic anemias, aberrant erythropoiesis, secondary anemia in nonhematolic disorders, anemias of chronic disease such as chronic renal failure; endocrine deficiency diseases; and/or erythrocytosis (e.g., polycythemia).

In a preferred embodiment, the disorder is a cancer, e.g., leukemic cancer, e.g., an erythroid leukemia, or a carcinoma, e.g., a renal carcinoma

In a preferred embodiment, the subject is a human.

In a preferred embodiment, the subject is an experimental animal, e.g., an animal model for an erythroid-associated disorder.

In a preferred embodiment, the method can further include treating the subject with a protein selected from the group consisting of G-CSF, GM-CSF, stem cell factor, Flt-3 ligand, IL-3, IL-4, thrombopoietin, and erythropoietin prior to assessing expression levels. Preferably, the protein is erythropoietin.

The invention also features a method of diagnosing a hematopoietic disorder, e.g., an erythroid-associated disorder, in a subject. The method includes evaluating the expression or activity of a 8834 nucleic acid or a 8834 polypeptide, such that, a difference in the level of 8834 nucleic acid or 8834 polypeptide relative to a normal subject or a cohort of normal subjects is indicative of the hematopoietic disorder.

In a preferred embodiment, the subject is a human.

In a preferred embodiment, the evaluating step occurs *in vitro* or *ex vivo*. For example, a sample, e.g., a blood sample, is obtained from the subject.

In a preferred embodiment, the evaluating step occurs *in vivo*. For example, by administering to the subject a detectably labeled agent that interacts with the 8834 nucleic acid or polypeptide, such that a signal is generated relative to the level of activity or expression of the 8834 nucleic acid or polypeptide.

In a preferred embodiment, the hematopoeitic disorder is an erythroid-associated disorder, e.g., an erythroid-associated disorder as described herein.

In yet another aspect, the invention features a method for identifying an agent, e.g., a agent, which modulates the activity of a 8834 polypeptide, e.g., a 8834 polypeptide as described herein, or the expression of a 8834 nucleic acid, e.g., a 8834 nucleic acid as described herein, including contacting the 8834 polypeptide or nucleic acid with a test agent (e.g., a test compound); and determining the effect of the test agent on the activity of the polypeptide or nucleic acid to thereby identify a agent which modulates the activity of the polypeptide or nucleic acid.

In a preferred embodiment, the activity of the 8834 polypeptide is protein phosphatase activity.

In a preferred embodiment, the activity of the 8834 polypeptide is hematopoiesis, e.g., erythropoiesis.

In a preferred embodiment, the activity of the 8834 polypeptide is proliferation, differentiation, and/or survival of a cell, e.g., a 8834-expressing cell, e.g., a hematopoietic cell (e.g., a bone marrow cell such as a CD34 positive cell, an erythroid cell, a megakaryocyte).

In preferred embodiments, the agent is a peptide, a phosphopeptide, a small molecule, e.g., a member of a combinatorial library, or an antibody, or any combination thereof.

In additional preferred embodiments, the agent is an antisense, a ribozyme, or a triple helix molecule, or an 8834 nucleic acid, or any combination thereof.

The terms "agent" and "compound" are used interchangeably herein.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Brief Description of the Drawings

Figure 1 depicts a cDNA sequence (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:2) of human 8843. The methionine-initiated open reading frame of human 8843 (without the 5' and 3' untranslated regions) until the end of SEQ ID NO:1 is shown also as coding sequence SEQ ID NO:3.

Figure 2 depicts a hydropathy plot of human 8843. Relative hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) are indicated by short vertical lines just below the hydropathy trace. The numbers corresponding to the amino acid sequence of human 8843 are indicated. Polypeptides of the invention include fragments which include: all or part of a hydrophobic sequence, i.e., a sequence above the dashed line, e.g., the sequence from about amino acid 9 to 25, from about 45 to 52, and from about 142 to 150, of SEQ ID NO:2; all or part of a hydrophilic sequence, i.e., a sequence below the dashed line, e.g., the sequence of from about amino acid 27 to 36, from about 70 to 78, and from about 151 to 160, of SEQ ID NO:2; a sequence which includes a Cys, or a glycosylation site.

Figure 3 depicts an alignment of the dual specificity phosphatase domain of human 8843 with a consensus amino acid sequence derived from a hidden Markov model (HMM) from PFAM. The upper sequence is the consensus amino acid sequence (SEQ ID NO:4), while the lower amino acid sequence corresponds to amino acids 37 to 185 of SEQ ID NO:2.

Figure 4 depicts an alignment of the dual specificity phosphatase domain of human 8843 with a consensus amino acid sequence derived from a hidden Markov model (HMM) in the SMART domain library. The upper sequence is the consensus amino acid sequence (SEQ ID NO:5), while the lower amino acid sequence corresponds to amino acids 37 to 185 of SEQ ID NO:2.

Figure 5 is a bar graph depicting relative 8843 mRNA expression as determined by the TaqMan assay on mRNA derived from a hematological human samples, e.g., erythroid cells (ery), megakaryocytes (meg), neutrophils, platelets, mast cells, and erythroid burst forming units.

Figure 6 is a bar graph depicting relative 8843 mRNA expression as determined by the TaqMan assay on mRNA derived from tissues including cord blood, fetal liver, and bone marrow, e.g., glycophorin A positive (low) bone marrow, and CD34+ bone marrow.

Figure 7 is a bar graph depicting relative 8843 mRNA expression as determined by the TaqMan assay on mRNA on from tissues including K562 cells, an erythroid-megakaryocyte line, and hepatic tissues.

Figure 8 is a bar graph depicting relative 8843 mRNA expression as determined by the TaqMan assay on mRNA derived from lung, fetal liver, and other tissues.

Detailed Description

The human 8843 sequence (Figure 1; SEQ ID NO:1), which is approximately 839 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 606 nucleotides, including the termination codon (nucleotides indicated as coding of SEQ ID NO:1 in Fig. 1; SEQ ID NO:3). The coding sequence encodes a 201 amino acid protein (SEQ ID NO:2).

Human 8843 contains the following regions or other structural features:

a dual specificity phosphatase domain (PFAM Accession Number PF00782) located at about amino acid residues 37 to 185 of SEQ ID NO:2;

a tyrosine specific protein phosphatase active site signature (Prosite PS00383), also termed "C-X_5-R" motif, located at about amino acid residues 130 to 142 of SEQ ID NO:2, including an active site cysteine at about amino acid 132 of SEQ ID NO:2, and an active site arginine at about amino acid 138 of SEQ ID NO:2;

a dual specificity phosphatase extended active site signature (VXVHCXXGXSRSXTXXXAY[LI]M; SEQ ID NO:8; Muda *et al.* (1996) *J Biol Chem* 271:27205) locate at about amino acid residues 128 to 158 of SEQ ID NO:2;

a VH1-like dual specificity phosphatase loop located at about amino acid residues 106 to 110 of SEQ ID NO:2, include a conserved general acid, aspartic acid at about residue 109 of SEQ ID NO:2;

one predicted N-glycosylation site (PS00001) at about amino acids 82 to 85 of SEQ ID NO:2;

one predicted protein kinase C phosphorylation sites (PS00005) at about amino acids 187 to 189 of SEQ ID NO:2;

four predicted casein kinase II phosphorylation sites (PS00006) located at about amino acids 70 to 73, 83 to 86, 98 to 101, and 154 to 157 of SEQ ID NO:2; and

one predicted N-myristylation sites (PS00008) from about amino acid 114 to 119 of SEQ ID NO:2.

For general information regarding PFAM identifiers, PS prefix and PF prefix domain identification numbers, refer to Sonnhammer et al. (1997) *Protein* 28:405-420 and http://www.psc.edu/general/ software/packages/pfam/pfam.html.

A plasmid containing the nucleotide sequence encoding human 8843 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number ______. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The 8843 protein contains a significant number of structural characteristics in common with members of the dual specificity phosphatase family. The term "family" when referring to the protein and nucleic acid molecules of the invention means two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin as well as other distinct proteins of human origin, or alternatively, can contain homologues of non-

human origin, e.g., rat or mouse proteins. Members of a family can also have common functional characteristics.

Dual specificity phosphatase proteins are characterized by a common fold. Dual specificity phosphatases are exemplified by the VH1 or vaccinia virus late H1 gene protein, which hydrolyzes both phosphotyrosine, phosphothreonine, and phosphoserine. VH1 catalytic activity is required for viral replication. A human homolog of VH1, VHR, has been identified. The three dimensional structure of this family is based on models from x-ray crystallographic data of protein tyrosine phosphatases, and human VHR. The VHR structure includes a core domain consisting of a five-stranded mixed β-sheet and six α-helices. This structure closely superimposes on the structure of phosphotyrosine protein phosphatases. However, dual specificity phosphatases lack the KNRY motif, and the N-terminal structures of tyrosine protein phosphatases which endow these enzymes with a deep active site specific for aryl phosphates. Thus, dual specificity phosphatases have a shallower active site relative to tyrosine protein phosphatases and can accommodate phosphoserine and phosphothreonine substrates. Even so dual specificity phosphatases can have a greater than 50-fold faster rate of phosphatase activity for phosphotyrosine substrates than phosphothreonine or phosphoserine substrates.

Similar to the broader class of phosphatases, dual specificity phosphatases have a highly conserved active site including three catalytic residues, a cysteine, an arginine, and an aspartic acid. The active site cysteine and arginine are found in the "C-X_5-R" motif of the tyrosine phosphatase signature (Prosite PS00383). This motif forms a binding pocket for three of the phosphate oxyanions. The cysteine acts as a nucleophile to accept the PO_3 group. The reaction transiently generates a phospho-cysteine intermediate before the phosphate is transferred to water. The active site arginine stabilizes the transition-state by hydrogen bonding to phosphate oxygens. In addition the histidine preceding the active site cysteine and the serine or threonine following the active site arginine are responsible for lowering the pK_a of the cysteine to stabilize a negative charge on the cysteine. The active site aspartic acid accelerates the reaction by donating a protein to generate an uncharged hydroxyl (for a review, see Fauman and Saper (1996) *Trends in Biochem.* 21:412).

An 8843 polypeptide can include a "dual specificity phosphatase catalytic domain" or regions homologous with a "dual specificity phosphatase domain".

As used herein, the term "dual specificity phosphatase domain" includes an amino acid sequence of about 80 to 220 amino acids, more preferably about 100 to 180 amino acid

residues, or about 130 to 160 amino acid residues in length and having a bit score for the alignment of the sequence to the dual specificity phosphatase domain (HMM) of at least 10, preferably 15, and more preferably 20. The dual specificity phosphatase catalytic domain (HMM) has been assigned the PFAM Accession Number PF00782 (http;//genome.wustl.edu/Pfam/.html). An alignment of the dual specificity phosphatase domain (amino acids 37 to 185 of SEQ ID NO:2) of human 8843 with a consensus amino acid sequence (SEQ ID NO:4) derived from a hidden Markov model is depicted in Figure 3, and a similar alignment with a consensus amino acid sequence (SEQ ID NO:5) derived from the SMART domain HMM model is depicted in Figure 4.

A dual specificity phosphatase domain preferably includes a perfect match to the Prosite tyrosine specific protein phosphatase active site signature (PS00383; [LIVMF]-H-C-x(2)-G-x(3)-[STC]-[STAGP]-x-[LIVMFY], wherein X is any amino acid and a number in parenthesis indicates the amino acid pattern is repeated that number of times; SEQ ID NO:6). Even more preferably, a dual specificity phosphatase includes the extended active site signature (VXVHCXXGXSRSXTXXXAY[LI]M; SEQ ID NO:8; Muda *et al.* (1996) *J Biol Chem* 271:27205). A dual specificity phosphatase domain also includes the conserved active site residues cysteine, arginine, and aspartic acid. The aspartic acid is preferably located in a loop region N-terminal to the active site signature.

In a preferred embodiment 8843 polypeptide or protein has a "dual specificity phosphatase domain" or a region which includes at least about 80 to 220 more preferably about 100 to 180 or 130 to 160, or about 148 amino acid residues in length and has at least about 50%, 60%, 70%, 80%, 90%, 95%, 99%, or 100% homology with a "dual specificity phosphatase domain," e.g., the dual specificity phosphatase domain of human 8843 (e.g., residues 37 to 185 of SEQ ID NO:2). In a preferred embodiment, the 8834 polypeptide has a tyrosine specific protein phosphatase active site signature located at about amino acids 130 to 142 of SEQ ID NO:2. The 8834 polypeptide also preferable has a conserved active site serine at about amino acid residue 132 of SEQ ID NO:2, a conserved active site arginine at about amino acid residue 138 of SEQ ID NO:2, and a conserved active site aspartic acid at about amino acid residue 109 of SEQ ID NO:2. Preferably, the active site aspartic acid is in an mobile loop, approximately 20 to 30, or preferably, 20 to 25 amino acids N-terminal to the active site cysteine.

To identify the presence of a "dual specificity phosphatase" domain in an 8843 protein sequence, and make the determination that a polypeptide or protein of interest has a

particular profile, the amino acid sequence of the protein can be searched against a database of HMMs (e.g., the Pfam database, release 2.1) using the default parameters (http://www.sanger.ac.uk/Software/Pfam/HMM_search). For example, the hmmsf program, which is available as part of the HMMER package of search programs, is a family specific default program for MILPAT0063 and a score of 15 is the default threshold score for determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in Sonhammer *et al.* (1997) *Proteins* 28(3):405-420 and a detailed description of HMMs can be found, for example, in Gribskov *et al.*(1990) *Meth. Enzymol.* 183:146-159; Gribskov *et al.*(1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh *et al.*(1994) *J. Mol. Biol.* 235:1501-1531; and Stultz *et al.*(1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference. A search was performed against the HMM database resulting in the identification of a "dual specificity phosphatase" domain in the amino acid sequence of human 8843 at about residues 37 to 185 of SEQ ID NO:2 (see Figure 1).

An 8843 molecule can further include: preferably at least one N-glycosylation site; preferably at least one protein kinase C phosphorylation sites; at least one, two, three, and preferably four casein kinase II phosphorylation sites; and preferably at least one N-myristylation site.

As used herein, a "8843 activity", "biological activity of 8843" or "functional activity of 8843", refers to an activity exerted by an 8843 protein, polypeptide or nucleic acid molecule on e.g., an 8843-responsive cell or on an 8843 substrate, e.g., a protein substrate, as determined *in vivo* or *in vitro*. In one embodiment, an 8843 activity is a direct activity, such as an association with an 8843 target molecule. A "target molecule" or "binding partner" is a molecule with which an 8843 protein binds or interacts in nature. an 8843 activity can also be an indirect activity, e.g., a cellular signaling activity mediated by interaction of the 8843 protein with an 8843 receptor. Based on the above-described sequence similarities, the 8843 molecules of the present invention are predicted to have similar biological activities as dual specificity phosphatase family members. For example, the 8843 proteins of the present invention can have one or more of the following activities: (1) catalyzing the removal of a phosphate group attached to a tyrosine residue in a protein; (2) catalyzing the removal of a phosphate group attached to a serine or threonine residue in a protein; (3) modulating an intracellular signaling pathway, e.g., a MAP kinase or ERK kinase pathway; (4) modulating cell differentiation, e.g., differentiation of erythroid

progenitor cells, such as, CD34+ progenitors; (5) modulating cell proliferation, e.g., proliferation erythroid progenitor cells; (6) inactivating cell surface growth factor receptors, e.g., tyrosine kinase receptors; or (7) modulating apoptosis, of a cell, e.g., a leukemic cell, (e.g., an erythroleukemia cell).

As the 8843 mRNA is found in hematopoietic cells, and in particular, in erythroid cell lineages (Figures 5-8), the molecules of the invention can be used to develop novel agents or compounds to treat and/or diagnose disorders involving aberrant activities of those cells e.g., hematopoietic and, in particular, erythroid disorders, as described below. For example, an 8843 polypeptide is expressed in CD34 positive cells, e.g., mobilized peripheral blood CD34+ cells, normal adult bone marrow CD34+ cells, cord blood CD34+ cells, normal adult bone marrow CD34+ cells, G-CSF-treated bone marrow CD34+ cells, and fetal liver CD34+ cells; and erythroid progenitor cells, e.g., bone marrow glycophorin A positive cells and erythropoietin treated erythroid burst forming units (BFUs) (Figures 5-8). 8843 mRNA is also expressed in hepatic cells, kidney, lung, and dermal cells, and thus diagnostic and therapeutic methods of using the molecules of the invention to treat/diagnose hepatic, kidney, lung, and dermal disorders are also contemplated by the present invention.

As used herein, the term " pluripotent hematopoietic stem cell" includes a cell that can give rise to a spleen colony forming unit (day 12 CFU-S), which, in turn, can give rise to progenitors of the granulocytic, monocytic, erythroid, megakaryocytic, and lymphoid lineages.

As used herein, a "CD34-positive cell" refers to a cell that expresses detectable levels of the CD34 antigen, preferably human CD34 antigen. The sequence for human CD34 is provided in SwissProt Accession Number P28906. The CD34 antigen is typically present on immature hematopoietic precursor cells and hematopoietic colony-forming cells in the bone marrow, including unipotent (CFU-GM, BFU-E) and pluripotent progenitors (CFU-GEMM, CFU-Mix and CFU-blast). The CD34 is also expressed on stromal cell precursors. Terminal deoxynucleotidyl transferase (TdT)-positive B- and T-lymphoid precursors in normal bone also are CD34+. The CD34 antigen is typically present on early myeloid cells that express the CD33 antigen, but lack the CD14 and CD15 antigens and on early erythroid cells that express the CD71 antigen and dimly express the CD45 antigen. The CD34 antigen is also found on capillary endothelial cells and approximately 1% of human thymocytes. Normal peripheral blood lymphocytes, monocytes, granulocytes and platelets do not express the CD34 antigen. CD34 antigen density is highest on early haematopoietic progenitor cells

and decreases as the cells mature. The antigen is undetectably on fully differentiated haematopoietic cells. Approximately 60% of acute B-lymphoid leukemia's and acute myeloid leukemia express the CD34 antigen. The antigen is not expressed on chronic lymphoid leukemia (B or T lineage) or lymphomas.

As the 8843 polypeptides of the invention may modulate 8843-mediated activities, they may be useful as of for developing novel diagnostic and therapeutic agents for 8843-mediated or related disorders, e.g., erythroid-associated disorders.

As used herein, the term "erythroid associated disorders" include disorders involving aberrant (increased or deficient) erythroblast proliferation, e.g., an erythroleukemia, and aberrant (increased or deficient) erythroblast differentiation, e.g., an anemia. Erythrocyte-associated disorders include anemias such as, for example, hemolytic anemias due to hereditary cell membrane abnormalities, such as hereditary spherocytosis, hereditary elliptocytosis, and hereditary pyropoikilocytosis; hemolytic anemias due to acquired cell membrane defects, such as paroxysmal nocturnal hemoglobinuria and spur cell anemia; hemolytic anemias caused by antibody reactions, for example to the RBC antigens, or antigens of the ABO system, Lewis system, Ii system, Rh system, Kidd system, Duffy system, and Kell system; methemoglobinemia; a failure of erythropoiesis, for example, as a result of aplastic anemia, pure red cell aplasia, myelodysplastic syndromes, sideroblastic anemias, and congenital dyserythropoietic anemia; secondary anemia in nonhematolic disorders, for example, as a result of chemotherapy (e.g., a drug- (e.g., chemotherapy-) induced anemia), alcoholism, or liver disease; anemia of chronic disease, such as chronic renal failure; and endocrine deficiency diseases. Preferably, the anemia is a drug- (e.g., chemotherapy-) induced anemia.

Compounds that modulate 8843 polypeptide or nucleic acid activity or expression can be used to treat anemias, in particular, anemias associated with cancer chemotherapy, chronic renal failure, malignancies, adult and juvenile rheumatoid arthritis, disorders of haemoglobin synthesis, prematurity, and zidovudine treatment of HIV infection. A subject receiving the treatment can be additionally treated with a second agent, e.g., erythropoietin, to futher ameliorate the condition. As used herein, the term "erythropoietin" or "EPO" refers to a glycoprotein produced in the kidney, which is the principal hormone responsible for stimulating red blood cell production (erythrogenesis). EPO stimulates the division and differentiation of committed erythroid progenitors in the bone marrow. Normal plasma erythropoietin levels range from 0.01 to 0.03 Units/mL, and can increase up to 100 to 1,000-

fold during hypoxia or anemia. Graber and Krantz, *Ann. Rev. Med.* 29:51 (1978); Eschbach and Adamson, *Kidney Intl.* 28:1 (1985). Recombinant human erythropoietin (rHuEpo or epoetin alfa) is commercially available as EPOGEN.RTM. (epoetin alfa, recombinant human erythropoietin) (Amgen Inc., Thousand Oaks, Calif.) and as PROCRIT.RTM. (epoetin alfa, recombinant human erythropoietin) (Ortho Biotech Inc., Raritan, N.J.).

Another example of an erythroid-associated disorder is erythrocytosis. Erythrocytosis, a disorder of red blood cell overproduction caused by excessive and/or ectopic erythropoietin production, can be caused by cancers, e.g., a renal cell cancer, a hepatocarcinoma, and a central nervous system cancer. Diseases associated with erythrocytosis include polycythemias, e.g., polycythemia vera, secondary polycythemia, and relative polycythemia.

Treatment, prevention and diagnosis of cancer or neoplastic disorders related to the erythroid lineage are also included in the present invention. Such neoplastic disorders are exemplified by erythroid leukemias, or leukemias of erythroid precursor cells, e.g., poorly differentiated acute leukemias such as erythroblastic leukemia and acute megakaryoblastic leukemia. Additional exemplary myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus, L. (1991) *Crit Rev. in Oncol./Hemotol.* 11:267-97). In particular, AML can include the uncontrolled proliferation of CD34+ cells such as AML subtypes M1 and M2, myeloblastic leukemias with and without maturation, and AML subtype M6, erythroleukemia (Di Guglielmo's disease). Additional neoplastic disorders include a myelodysplastic syndrome or preleukemic disorder, e.g., oligoblastic leukemia, smoldering leukemia. Additional cancers of the erythroid lineage include erythroblastosis, and other relevant diseases of the bone marrow.

As used herein, the terms “cancer”, "hyperproliferative" and "neoplastic" refer to cells having the capacity for autonomous growth, i.e., an abnormal state or condition characterized by rapidly proliferating cell growth.

The term "leukemia" or "leukemic cancer" is intended to have its clinical meaning, namely, a neoplastic disease in which white corpuscle maturation is arrested at a primitive stage of cell development. The disease is characterized by an increased number of leukemic blast cells in the bone marrow, and by varying degrees of failure to produce normal hematopoietic cells. The condition may be either acute or chronic. Leukemias are further typically categorized as being either lymphocytic i.e., being characterized by cells which

have properties in common with normal lymphocytes, or myelocytic (or myelogenous), i.e., characterized by cells having some characteristics of normal granulocytic cells. Acute lymphocytic leukemia ("ALL") arises in lymphoid tissue, and ordinarily first manifests its presence in bone marrow. Acute myelocytic leukemia ("AML") arises from bone marrow hematopoietic stem cells or their progeny. The term acute myelocytic leukemia subsumes several subtypes of leukemia: myeloblastic leukemia, promyelocytic leukemia, and myelomonocytic leukemia. In addition, leukemias with erythroid or megakaryocytic properties are considered myelogenous leukemias as well.

The 8843 protein, fragments thereof, and derivatives and other variants of the sequence in SEQ ID NO:2 thereof are collectively referred to as "polypeptides or proteins of the invention" or "8843 polypeptides or proteins". Nucleic acid molecules encoding such polypeptides or proteins are collectively referred to as "nucleic acids of the invention" or "8843 nucleic acids." 8843 molecules refer to 8843 nucleic acids, polypeptides, and antibodies.

As used herein, the term "nucleic acid molecule" includes DNA molecules (e.g., a cDNA or genomic DNA) and RNA molecules (e.g., an mRNA) and analogs of the DNA or RNA generated, e.g., by the use of nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated or purified nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules, which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and/or 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of 5' and/or 3' nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

As used herein, the term "hybridizes under stringent conditions" describes conditions for hybridization and washing. Stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. A preferred, example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50°C. Another example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 55°C. A further example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C. Preferably, stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. Particularly preferred stringency conditions (and the conditions that should be used if the practitioner is uncertain about what conditions should be applied to determine if a molecule is within a hybridization limitation of the invention) are 0.5M Sodium Phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 or SEQ ID NO:3, corresponds to a naturally-occurring nucleic acid molecule.

As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding an 8843 protein, preferably a mammalian 8843 protein, and can further include non-coding regulatory sequences, and introns.

An "isolated" or "purified" polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. In one embodiment, the language "substantially free" means preparation of 8843 protein having less than about 30%, 20%, 10% and more preferably 5% (by dry weight), of non-8843 protein (also referred to herein as a "contaminating protein"),

or of chemical precursors or non-8843 chemicals. When the 8843 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The invention includes isolated or purified preparations of at least 0.01, 0.1, 1.0, and 10 milligrams in dry weight.

A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of 8843 (e.g., the sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____) without abolishing or more preferably, without substantially altering a biological activity, whereas an "essential" amino acid residue results in such a change. For example, amino acid residues that are conserved among the polypeptides of the present invention, e.g., those present in the dual specificity phosphatase domain, are predicted to be particularly unamenable to alteration.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in an 8843 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an 8843 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for 8843 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1 or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

As used herein, a "biologically active portion" of an 8843 protein includes a fragment of an 8843 protein which participates in an interaction between an 8843 molecule

and a non-8843 molecule. Biologically active portions of an 8843 protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the 8843 protein, e.g., the amino acid sequence shown in SEQ ID NO:2, which include less amino acids than the full length 8843 proteins, and exhibit at least one activity of an 8843 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the 8843 protein, e.g., protein phosphatase activity. A biologically active portion of an 8843 protein can be a polypeptide which is, for example, 10, 25, 50, 100, 200 or more amino acids in length. Biologically active portions of an 8843 protein can be used as targets for developing agents, which modulate an 8843 mediated activity, e.g., protein phosphatase activity.

Particular 8843 polypeptides of the present invention have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2. The term "sufficiently identical" or "substantially identical" is used herein to refer to a first amino acid or nucleotide sequence that contains a sufficient or minimum number of identical or equivalent (e.g., with a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have a common structural domain or common functional activity. For example, amino acid or nucleotide sequences that contain a common structural domain having at least about 60%, or 65% identity, likely 75% identity, more likely 85%, 90%. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity are defined herein as sufficiently or substantially identical.

Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence (e.g., when aligning a second sequence to the 8843 amino acid sequence of SEQ ID NO:2 having 149 amino acid residues, at least 45, preferably at least 60 , more preferably at least 75, even more preferably at least 90, and even more preferably at least 104, 120, or 134 amino acid residues are aligned). The amino

acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a sequence identity or homology limitation of the invention) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to 8843 nucleic acid molecules

of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to 8843 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

"Misexpression or aberrant expression", as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes: expression at non-wild type levels, i.e., over or under expression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-transitional modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus.

"Subject", as used herein, can refer to a mammal, e.g., a human, or to an experimental or animal or disease model. The subject can also be a non-human animal, e.g., a horse, cow, goat, or other domestic animal.

A "purified preparation of cells", as used herein, refers to, in the case of plant or animal cells, an in vitro preparation of cells and not an entire intact plant or animal. In the case of cultured cells or microbial cells, it consists of a preparation of at least 10% and more preferably 50% of the subject cells.

Various aspects of the invention are described in further detail below.

<u>Isolated Nucleic Acid Molecules</u>

In one aspect, the invention provides, an isolated or purified, nucleic acid molecule that encodes an 8843 polypeptide described herein, e.g., a full length 8843 protein or a fragment thereof, e.g., a biologically active portion of 8843 protein. Also included is a nucleic acid fragment suitable for use as a hybridization probe, which can be used, e.g., to a

identify nucleic acid molecule encoding a polypeptide of the invention, 8843 mRNA, and fragments suitable for use as primers, e.g., PCR primers for the amplification or mutation of nucleic acid molecules.

In one embodiment, an isolated nucleic acid molecule of the invention includes the nucleotide sequence shown in SEQ ID NO:1, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion of any of these nucleotide sequences. In one embodiment, the nucleic acid molecule includes sequences encoding the human 8843 protein (i.e., "the coding region" of SEQ ID NO:1, as shown in SEQ ID NO:3), as well as 5' untranslated sequences. Alternatively, the nucleic acid molecule can include only the coding region of SEQ ID NO:1 (e.g., SEQ ID NO:3) and, e.g., no flanking sequences which normally accompany the subject sequence. In another embodiment, the nucleic acid molecule encodes a sequence corresponding to a fragment of the protein from about amino acid 25 to 190, or about 37 to 185 of SEQ ID NO:2.

In another embodiment, an isolated nucleic acid molecule of the invention includes a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion of any of these nucleotide sequences. In other embodiments, the nucleic acid molecule of the invention is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, thereby forming a stable duplex.

In one embodiment, an isolated nucleic acid molecule of the present invention includes a nucleotide sequence which is at least about 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homologous to the entire length of the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3, or the entire length of the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion, preferably of the same length, of any of these nucleotide sequences.

<u>8843 Nucleic Acid Fragments</u>

A nucleic acid molecule of the invention can include only a portion of the nucleic acid sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the

plasmid deposited with ATCC as Accession Number _____. For example, such a nucleic acid molecule can include a fragment which can be used as a probe or primer or a fragment encoding a portion of an 8843 protein, e.g., an immunogenic or biologically active portion of an 8843 protein. A fragment can comprise those nucleotides of SEQ ID NO:1, which encode a dual specificity phosphatase domain of human 8843. The nucleotide sequence determined from the cloning of the 8843 gene allows for the generation of probes and primers designed for use in identifying and/or cloning other 8843 family members, or fragments thereof, as well as 8843 homologues, or fragments thereof, from other species.

In another embodiment, a nucleic acid includes a nucleotide sequence that includes part, or all, of the coding region and extends into either (or both) the 5' or 3' noncoding region. Other embodiments include a fragment which includes a nucleotide sequence encoding an amino acid fragment described herein. Nucleic acid fragments can encode a specific domain or site described herein or fragments thereof, particularly fragments thereof which are at least 100, 150, or 200 amino acids in length. Fragments also include nucleic acid sequences corresponding to specific amino acid sequences described above or fragments thereof. Nucleic acid fragments should not to be construed as encompassing those fragments that may have been disclosed prior to the invention.

A nucleic acid fragment can include a sequence corresponding to a domain, region, or functional site described herein. A nucleic acid fragment can also include one or more domain, region, or functional site described herein. Thus, for example, an 8843 nucleic acid fragment can include a sequence corresponding to a dual specificity phosphatase domain.

8843 probes and primers are provided. Typically a probe/primer is an isolated or purified oligonucleotide. The oligonucleotide typically includes a region of nucleotide sequence that hybridizes under stringent conditions to at least about 7, 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense or antisense sequence of SEQ ID NO:1 or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1 or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

In a preferred embodiment the nucleic acid is a probe which is at least 5 or 10, and less than 200, more preferably less than 100, or less than 50, base pairs in length. It should be identical, or differ by 1, or less than in 5 or 10 bases, from a sequence disclosed herein.

If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

A probe or primer can be derived from the sense or anti-sense strand of a nucleic acid which encodes: a dual specificity phosphatase domain, e.g., about amino acids 25 to 201, or about 37 to 185 of SEQ ID NO:2. In another embodiment a set of primers is provided, e.g., primers suitable for use in a PCR, which can be used to amplify a selected region of an 8843 sequence, e.g., a domain, region, site or other sequence described herein. The primers should be at least 5, 10, or 50 base pairs in length and less than 100, or less than 200, base pairs in length. The primers should be identical, or differs by one base from a sequence disclosed herein or from a naturally occurring variant. For example, primers suitable for amplifying all or a portion of any of the following regions are provided: a dual specificity phosphatase domain from about amino acid 25 to 201, or from 37 to 185 of SEQ ID NO:2.

A nucleic acid fragment can encode an epitope bearing region of a polypeptide described herein.

A nucleic acid fragment encoding a "biologically active portion of an 8843 polypeptide" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, which encodes a polypeptide having an 8843 biological activity (e.g., the biological activities of the 8843 proteins are described herein), expressing the encoded portion of the 8843 protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the 8843 protein. For example, a nucleic acid fragment encoding a biologically active portion of 8843 includes a dual specificity phosphatase domain, e.g., amino acid residues about 37 to 185 of SEQ ID NO:2. A nucleic acid fragment encoding a biologically active portion of an 8843 polypeptide, may comprise a nucleotide sequence which is greater than 300 or more nucleotides in length.

In preferred embodiments, a nucleic acid includes a nucleotide sequence which is about 300, 400, 500, 600, 700, 800, or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

8843 Nucleic Acid Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. Such differences can be due to degeneracy of the genetic code (and result in a nucleic acid which encodes the same 8843 proteins as those encoded by the nucleotide sequence disclosed herein. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence which differs, by at least 1, but less than 5, 10, 20, 50, or 100 amino acid residues that shown in SEQ ID NO:2. If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

Nucleic acids of the inventor can be chosen for having codons, which are preferred, or non preferred, for a particular expression system. E.g., the nucleic acid can be one in which at least one colon, at preferably at least 10%, or 20% of the codons has been altered such that the sequence is optimized for expression in *E. coli*, yeast, human, insect, or CHO cells.

Nucleic acid variants can be naturally occurring, such as allelic variants (same locus), homologs (different locus), and orthologs (different organism) or can be non naturally occurring. Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded product).

In a preferred embodiment, the nucleic acid differs from that of SEQ ID NO: 1 or 3, or the sequence in ATCC Accession Number ___, e.g., as follows: by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 1%, 5%, 10% or 20% of the in the subject nucleic acid. If necessary for this analysis the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

Orthologs, homologs, and allelic variants can be identified using methods known in the art. These variants comprise a nucleotide sequence encoding a polypeptide that is 50%, at least about 55%, typically at least about 70-75%, more typically at least about 80-85%, and most

typically at least about 90-95% or more identical to the nucleotide sequence shown in SEQ ID NO:2 or a fragment of this sequence. Such nucleic acid molecules can readily be identified as being able to hybridize under stringent conditions, to the nucleotide sequence shown in SEQ ID NO 2 or a fragment of the sequence. Nucleic acid molecules corresponding to orthologs, homologs, and allelic variants of the 8843 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the 8843 gene.

Preferred variants include those that are correlated with protein phosphatase activity.

Allelic variants of 8843, e.g., human 8843, include both functional and non-functional proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the 8843 protein within a population that maintain the ability to hydrolyze the phosphate of phosphorylated proteins. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein. Non-functional allelic variants are naturally-occurring amino acid sequence variants of the 8843, e.g., human 8843, protein within a population that do not have the ability to to hydrolyze the phosphate of phosphorylated proteins. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion, or premature truncation of the amino acid sequence of SEQ ID NO:2, or a substitution, insertion, or deletion in critical residues or critical regions of the protein.

Moreover, nucleic acid molecules encoding other 8843 family members and, thus, which have a nucleotide sequence which differs from the 8843 sequences of SEQ ID NO:1 or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ are intended to be within the scope of the invention.

<u>Antisense Nucleic Acid Molecules, Ribozymes and Modified 8843 Nucleic Acid Molecules</u>

In another aspect, the invention features, an isolated nucleic acid molecule which is antisense to 8843. An "antisense" nucleic acid can include a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. The antisense nucleic acid can be complementary to an entire 8843 coding strand, or to only a portion thereof (e.g., the coding region of human 8843 corresponding to SEQ ID NO:3). In another embodiment, the antisense nucleic acid molecule is antisense to a

"noncoding region" of the coding strand of a nucleotide sequence encoding 8843 (e.g., the 5' and 3' untranslated regions).

An antisense nucleic acid can be designed such that it is complementary to the entire coding region of 8843 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of 8843 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of 8843 mRNA, e.g., between the -10 and +10 regions of the target gene nucleotide sequence of interest. An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more nucleotides in length.

An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. The antisense nucleic acid also can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject (e.g., by direct injection at a tissue site), or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an 8843 protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier et al. (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. A ribozyme having specificity for an 8843-encoding nucleic acid can include one or more sequences complementary to the nucleotide sequence of an 8843 cDNA disclosed herein (i.e., SEQ ID NO:1 or SEQ ID NO:3), and a sequence having known catalytic sequence responsible for mRNA cleavage (see U.S. Pat. No. 5,093,246 or Haselhoff and Gerlach (1988) *Nature* 334:585-591). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an 8843-encoding mRNA. See, e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742. Alternatively, 8843 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

8843 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the 8843 (e.g., the 8843 promoter and/or enhancers) to form triple helical structures that prevent transcription of the 8843 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. et al. (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15. The potential sequences that can be targeted for triple helix formation can be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

The invention also provides detectably labeled oligonucleotide primer and probe molecules. Typically, such labels are chemiluminescent, fluorescent, radioactive, or colorimetric.

An 8843 nucleic acid molecule can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. et al. (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acid" or "PNA" refers to a nucleic acid mimic, e.g., a DNA mimic, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of a PNA can allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. et al. (1996) *supra*; Perry-O'Keefe et al. Proc. Natl. Acad. Sci. 93: 14670-675.

PNAs of 8843 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of 8843 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. et al. (1996) *supra*; Perry-O'Keefe *supra*).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol et al. (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

The invention also includes molecular beacon oligonucleotide primer and probe molecules having at least one region which is complementary to an 8843 nucleic acid of the invention, two complementary regions one having a fluorophore and one a quencher such

that the molecular beacon is useful for quantitating the presence of the 8843 nucleic acid of the invention in a sample. Molecular beacon nucleic acids are described, for example, in Lizardi et al., U.S. Patent No. 5,854,033; Nazarenko et al., U.S. Patent No. 5,866,336, and Livak et al., U.S. Patent 5,876,930.

Isolated 8843 Polypeptides

In another aspect, the invention features, an isolated 8843 protein, or fragment, e.g., a biologically active portion, for use as immunogens or antigens to raise or test (or more generally to bind) anti-8843 antibodies. 8843 protein can be isolated from cells or tissue sources using standard protein purification techniques. 8843 protein or fragments thereof can be produced by recombinant DNA techniques or synthesized chemically.

Polypeptides of the invention include those which arise as a result of the existence of multiple genes, alternative transcription events, alternative RNA splicing events, and alternative translational and post-translational events. The polypeptide can be expressed in systems, e.g., cultured cells, which result in substantially the same post-translational modifications present when expressed the polypeptide is expressed in a native cell, or in systems which result in the alteration or omission of post-translational modifications, e.g., glycosylation or cleavage, present when expressed in a native cell.

In a preferred embodiment, an 8843 polypeptide has one or more of the following characteristics:

(i) it has the ability to hydrolyze the phosphate of phosphorylated proteins, i.e., it has protein phosphatase activity, e.g., for phosphotyrosine, phosphoserine, and phosphothreonine substrates;

(ii) it has a molecular weight, e.g., a deduced molecular weight, preferably ignoring any contribution of post translational modifications, amino acid composition or other physical characteristic of SEQ ID NO:2;

(iii) it has an overall sequence similarity of at least 90, 95, 96, 97, 98, 99, 100%, with a polypeptide of SEQ ID NO:2;

(iv) it can be found in erythroid cells, especially erythroid progenitor cells, e.g., CD34+ progenitor cells, bone marrow cells (CD34+ and glycophorin A positive), erythroblasts, erythropoietin treated erythroid cells;

(v) it can be found in a CD34+ cell;

(v) it has a dual specificity phosphatase domain which is preferably about 70%, 80%, 90% or 95% with amino acid residues about 37 to 185 of SEQ ID NO:2;

(vii) it has an active site cysteine at about residue 132 of SEQ ID NO:2, an active site arginine at about residue 138 of SEQ ID NO:2, and an active site aspartic acid at about residue 109 of SEQ ID NO:2;

(viii) it has a dual specificity phosphatase extended active site signature (VXVHCXXGXSRSXTXXXAY[LI]M; SEQ ID NO:8; Muda *et al.* (1996) *J Biol Chem* 271:27205) locate at about amino acid residues 128 to 158 of SEQ ID NO:2; in addition to a tyrosine specific protein phosphatase active site signature (Prosite PS00383) located at about amino acid residues 130 to 142 of SEQ ID NO:2;

(ix) it has a VH1-like dual specificity phosphatase loop located at about amino acid residues 106 to 110 of SEQ ID NO:2, include a conserved general acid, aspartic acid at about residue 109 of SEQ ID NO:2;

(x) it has one predicted N-glycosylation site (PS00001) at about amino acids 82 to 85 of SEQ ID NO:2;

(xi) it has one predicted protein kinase C phosphorylation sites (PS00005) at about amino acids 187 to 189 of SEQ ID NO:2;

(xii) it has four predicted casein kinase II phosphorylation sites (PS00006) located at about amino acids 70 to 73, 83 to 86, 98 to 101, and 154 to 157 of SEQ ID NO:2; or

(xiii) it has one predicted N-myristylation sites (PS00008) from about amino acid 114 to 119 of SEQ ID NO:2.

In a preferred embodiment the 8843 protein, or fragment thereof, differs from the corresponding sequence in SEQ ID:2. In one embodiment it differs by at least one but by less than 15, 10 or 5 amino acid residues. In another it differs from the corresponding sequence in SEQ ID NO:2 by at least one residue but less than 20%, 15%, 10% or 5% of the residues in it differ from the corresponding sequence in SEQ ID NO:2. (If this comparison requires alignment the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.) The differences are, preferably, differences or changes at a non essential residue or a conservative substitution. In a preferred embodiment the differences are not in the dual specificity phosphatase domain. In another preferred embodiment one or more differences are in the dual specificity phosphatase domain.

Other embodiments include a protein that contain one or more changes in amino acid sequence, e.g., a change in an amino acid residue which is not essential for activity. Such 8843 proteins differ in amino acid sequence from SEQ ID NO:2, yet retain biological activity.

In one embodiment, the protein includes an amino acid sequence at least about 80%, 85%, 90%, 95%, 98%, 99%, or more homologous to SEQ ID NO:2.

A 8843 protein or fragment is provided which varies from the sequence of SEQ ID NO.2 in regions defined by amino acids about 37 to 185 of SEQ ID NO.2 by at least one but by less than 15, 10 or 5 amino acid residues in the protein or fragment but which does not differ from SEQ ID NO.2 in regions defined by amino acids about 37 to 185 of SEQ ID NO.2. (If this comparison requires alignment the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.) In some embodiments the difference is at a non essential residue or is a conservative substitution, while in others the difference is at an essential residue or is a non conservative substitution.

In one embodiment, a biologically active portion of an 8843 protein includes a dual specificity phosphatase domain. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native 8843 protein.

In a preferred embodiment, the 8843 protein has an amino acid sequence shown in SEQ ID NO:2. In other embodiments, the 8843 protein is substantially identical to SEQ ID NO:2. In yet another embodiment, the 8843 protein is substantially identical to SEQ ID NO:2 and retains the functional activity of the protein of SEQ ID NO:2, as described in detail in the subsections above.

8843 Chimeric or Fusion Proteins

In another aspect, the invention provides 8843 chimeric or fusion proteins. As used herein, an 8843 "chimeric protein" or "fusion protein" includes an 8843 polypeptide linked to a non-8843 polypeptide. A "non-8843 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the 8843 protein, e.g., a protein which is different from the 8843 protein and which is derived from the same or a different organism. The 8843 polypeptide of the fusion protein can correspond to all or a portion e.g., a fragment described herein of an 8843 amino acid

sequence. In a preferred embodiment, an 8843 fusion protein includes at least one (or two) biologically active portion of an 8843 protein. The non-8843 polypeptide can be fused to the N-terminus or C-terminus of the 8843 polypeptide.

The fusion protein can include a moiety, which has a high affinity for a ligand. For example, the fusion protein can be a GST-8843 fusion protein in which the 8843 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant 8843. Alternatively, the fusion protein can be an 8843 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of 8843 can be increased through use of a heterologous signal sequence.

Fusion proteins can include all or a part of a serum protein, e.g., an IgG constant region, or human serum albumin.

The 8843 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The 8843 fusion proteins can be used to affect the bioavailability of an 8843 substrate. 8843 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding an 8843 protein; (ii) mis-regulation of the 8843 gene; and (iii) aberrant post-translational modification of an 8843 protein.

Moreover, the 8843-fusion proteins of the invention can be used as immunogens to produce anti-8843 antibodies in a subject, to purify 8843 ligands and in screening assays to identify molecules which inhibit the interaction of 8843 with an 8843 substrate.

Expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). an 8843-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the 8843 protein.

Variants of 8843 Proteins

In another aspect, the invention also features a variant of an 8843 polypeptide, e.g., which functions as an agonist (mimetics) or as an antagonist. Variants of the 8843 proteins can be generated by mutagenesis, e.g., discrete point mutation, the insertion or deletion of sequences or the truncation of an 8843 protein. An agonist of the 8843 proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of an 8843 protein. An antagonist of an 8843 protein can inhibit one or more of the activities of the naturally occurring form of the 8843 protein by, for example, competitively

modulating an 8843-mediated activity of an 8843 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Preferably, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 8843 protein.

Variants of an 8843 protein can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of an 8843 protein for agonist or antagonist activity.

Libraries of fragments e.g., N terminal, C terminal, or internal fragments, of an 8843 protein coding sequence can be used to generate a variegated population of fragments for screening and subsequent selection of variants of an 8843 protein.

Variants in which a cysteine residues is added or deleted or in which a residue which is glycosylated is added or deleted are particularly preferred.

Methods for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify 8843 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave et al. (1993) *Protein Engineering* 6(3):327-331).

Cell based assays can be exploited to analyze a variegated 8843 library. For example, a library of expression vectors can be transfected into a cell line, e.g., a cell line, which ordinarily responds to 8843 in a substrate-dependent manner. The transfected cells are then contacted with 8843 and the effect of the expression of the mutant on signaling by the 8843 substrate can be detected, e.g., by measuring protein phosphatase activity. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of signaling by the 8843 substrate, and the individual clones further characterized.

In another aspect, the invention features a method of making an 8843 polypeptide, e.g., a peptide having a non-wild type activity, e.g., an antagonist, agonist, or super agonist of a naturally occurring 8843 polypeptide, e.g., a naturally occurring 8843 polypeptide. The method includes: altering the sequence of an 8843 polypeptide, e.g., altering the sequence , e.g., by substitution or deletion of one or more residues of a non-conserved region, a domain or residue disclosed herein, and testing the altered polypeptide for the desired activity.

In another aspect, the invention features a method of making a fragment or analog of an 8843 polypeptide a biological activity of a naturally occurring 8843 polypeptide. The method includes: altering the sequence, e.g., by substitution or deletion of one or more residues, of an 8843 polypeptide, e.g., altering the sequence of a non-conserved region, or a domain or residue described herein, and testing the altered polypeptide for the desired activity.

Anti-8843 Antibodies

In another aspect, the invention provides an anti-8843 antibody. The term "antibody" as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, i.e., an antigen-binding portion. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin.

The antibody can be a polyclonal, monoclonal, recombinant, e.g., a chimeric or humanized, fully human, non-human, e.g., murine, or single chain antibody. In a preferred embodiment it has effector function and can fix complement. The antibody can be coupled to a toxin or imaging agent.

A full-length 8843 protein or, antigenic peptide fragment of 8843 can be used as an immunogen or can be used to identify anti-8843 antibodies made with other immunogens, e.g., cells, membrane preparations, and the like. The antigenic peptide of 8843 should include at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 and encompasses an epitope of 8843. Preferably, the antigenic peptide includes at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Fragments of 8843 which include residues about 9 to 25, about 45 to 52, or about 142 to 150 of SEQ ID NO.2 can be used to make, e.g., used as immunogens or used to characterize the specificity of an antibody, antibodies against hydrophobic regions of the 8843 protein. Similarly, a fragment of 8843 which include residues about 27 to 36, about 70 to 78, or about 151 to 160 of SEQ ID NO.2 can be used to make an antibody against a hydrophilic region of the 8843 protein; a fragment of 8843 which include residues about 25 to 201, or about 37 to 185 of SEQ ID NO.2 can be used to make an antibody against the dual specificity phosphatase region of the 8843 protein.

Antibodies reactive with, or specific for, any of these regions, or other regions or domains described herein are provided.

Preferred epitopes encompassed by the antigenic peptide are regions of 8843 are located on the surface of the protein, e.g., hydrophilic regions, as well as regions with high antigenicity. For example, an Emini surface probability analysis of the human 8843 protein sequence can be used to indicate the regions that have a particularly high probability of being localized to the surface of the 8843 protein and are thus likely to constitute surface residues useful for targeting antibody production.

In a preferred embodiment the antibody binds an epitope on any domain or region on 8843 proteins described herein.

Chimeric, humanized, but most preferably, completely human antibodies are desirable for applications which include repeated administration, e.g., therapeutic treatment (and some diagnostic applications) of human patients.

The anti-8843 antibody can be a single chain antibody. A single-chain antibody (scFV) may be engineered (see, for example, Colcher, D., et al. *Ann N Y Acad Sci* 1999 Jun 30;880:263-80; and Reiter, Y. *Clin Cancer Res* 1996 Feb;2(2):245-52). The single chain antibody can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target 8843 protein.

In a preferred embodiment, the antibody has reduced or no ability to bind an Fc receptor. For example., it is a isotype or subtype, fragment or other mutant, which does not support binding to an Fc receptor, e.g., it has a mutagenized or deleted Fc receptor binding region.

An anti-8843 antibody (e.g., monoclonal antibody) can be used to isolate 8843 by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, an anti-8843 antibody can be used to detect 8843 protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein. Anti-8843 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance (i.e., antibody labelling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase;

examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

Recombinant Expression Vectors, Host Cells and Genetically Engineered Cells

In another aspect, the invention includes, vectors, preferably expression vectors, containing a nucleic acid encoding a polypeptide described herein. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked and can include a plasmid, cosmid or viral vector. The vector can be capable of autonomous replication or it can integrate into a host DNA. Viral vectors include, e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses.

A vector can include an 8843 nucleic acid in a form suitable for expression of the nucleic acid in a host cell. Preferably the recombinant expression vector includes one or more regulatory sequences operatively linked to the nucleic acid sequence to be expressed. The term "regulatory sequence" includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence, as well as tissue-specific regulatory and/or inducible sequences. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or polypeptides, including fusion proteins or polypeptides, encoded by nucleic acids as described herein (e.g., 8843 proteins, mutant forms of 8843 proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of 8843 proteins in prokaryotic or eukaryotic cells. For example, polypeptides of the invention can be expressed in *E. coli*, insect cells (e.g., using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San

Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be used in 8843 activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for 8843 proteins. In a preferred embodiment, a fusion protein expressed in a retroviral expression vector of the present invention can be used to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six weeks).

To maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada et al., (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

The 8843 expression vector can be a yeast expression vector, a vector for expression in insect cells, e.g., a baculovirus expression vector or a vector suitable for expression in mammalian cells.

When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji et al. (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example, the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. Regulatory sequences (e.g., viral promoters and/or enhancers) operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the constitutive, tissue specific or cell type specific expression of antisense RNA in a variety of cell types. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect the invention provides a host cell which includes a nucleic acid molecule described herein, e.g., an 8843 nucleic acid molecule within a recombinant expression vector or an 8843 nucleic acid molecule containing sequences which allow it to

homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, an 8843 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into host cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation.

A host cell of the invention can be used to produce (i.e., express) an 8843 protein. Accordingly, the invention further provides methods for producing an 8843 protein using the host cells of the invention. In one embodiment, the method includes culturing the host cell of the invention (into which a recombinant expression vector encoding an 8843 protein has been introduced) in a suitable medium such that an 8843 protein is produced. In another embodiment, the method further includes isolating an 8843 protein from the medium or the host cell.

In another aspect, the invention features, a cell or purified preparation of cells which include an 8843 transgene, or which otherwise misexpress 8843. The cell preparation can consist of human or non human cells, e.g., rodent cells, e.g., mouse or rat cells, rabbit cells, or pig cells. In preferred embodiments, the cell or cells include an 8843 transgene, e.g., a heterologous form of an 8843, e.g., a gene derived from humans (in the case of a non-human cell). The 8843 transgene can be misexpressed, e.g., overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene which misexpress an endogenous 8843, e.g., a gene the expression of which is disrupted, e.g., a knockout. Such cells can serve as a model for studying disorders which are related to mutated or mis-expressed 8843 alleles or for use in drug screening.

In another aspect, the invention features, a human cell, e.g., a hematopoietic stem cell, transformed with nucleic acid which encodes a subject 8843 polypeptide.

Also provided are cells, preferably human cells, e.g., human hematopoietic or fibroblast cells, in which an endogenous 8843 is under the control of a regulatory sequence that does not normally control the expression of the endogenous 8843 gene. The expression characteristics of an endogenous gene within a cell, e.g., a cell line or microorganism, can be modified by inserting a heterologous DNA regulatory element into the genome of the cell such that the inserted regulatory element is operably linked to the endogenous 8843 gene. For example, an endogenous 8843 gene which is "transcriptionally silent," e.g., not normally expressed, or expressed only at very low levels, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell. Techniques such as targeted homologous recombinations, can be used to insert the heterologous DNA as described in, e.g., Chappel, US 5,272,071; WO 91/06667, published in May 16, 1991.

Transgenic Animals

The invention provides non-human transgenic animals. Such animals are useful for studying the function and/or activity of an 8843 protein and for identifying and/or evaluating modulators of 8843 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA or a rearrangement, e.g., a deletion of endogenous chromosomal DNA, which preferably is integrated into or occurs in the genome of the cells of a transgenic animal. A transgene can direct the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal, other transgenes, e.g., a knockout, reduce expression. Thus, a transgenic animal can be one in which an endogenous 8843 gene has been altered by, e.g., by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a transgene of the invention to direct expression of an

8843 protein to particular cells. A transgenic founder animal can be identified based upon the presence of an 8843 transgene in its genome and/or expression of 8843 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding an 8843 protein can further be bred to other transgenic animals carrying other transgenes.

8843 proteins or polypeptides can be expressed in transgenic animals or plants, e.g., a nucleic acid encoding the protein or polypeptide can be introduced into the genome of an animal. In preferred embodiments the nucleic acid is placed under the control of a tissue specific promoter, e.g., a milk or egg specific promoter, and recovered from the milk or eggs produced by the animal. Suitable animals are mice, pigs, cows, goats, and sheep.

The invention also includes a population of cells from a transgenic animal, as discussed, e.g., below.

8834 Molecules In Erythropoietin-Responsive Cells

As 8834 molecules are abundantly expressed in erythroid progenitor cells, 8834 molecules can modulate the differentiative process undergone by progenitors to produce erythrocytes. The final stages of erythroid differentiation are regulated by erythropoietin, which is an extremely potent soluble polypeptide regulator. Erythropoietin can be administered at approximately 50 units per kilogram of body weight every other day in order to increase red blood cell mass. Erythropoietin can be used to treat anemias, especially anemia due to chronic renal failure. Alternatively, it can be used to increase patient hemocrit prior to operations.

In normal subjects, the peritubular intestinal cells of the kidney produce erythropoietin in response to lowered oxygen levels. However, in pathological states, such as cancers, especially renal cancers, erythropoietin can be overproduced resulting in erythrocytosis due to excess red blood cell production.

Contemplated by the current invention are methods of modulating the erythropoietin-induce differentiative response by agonizing or antagonizing 8834 polypeptides and nucleic acids. In one embodiment, 8834 dual specificity phosphatase can increase cellular signalling in response to erythropoietin. In another embodiment, it can antagonize erythropoietin-induce phosphorylation signals. It will be readily apparent to one skilled in the art, that 8834 activity can be modulated by altering 8834 expression levels, or by small molecules, or proteins which bind to 8834 polypeptides in order alter a cell's response to erythropoietin.

Uses

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (e.g., therapeutic and prophylactic).

The isolated nucleic acid molecules of the invention can be used, for example, to express an 8843 protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect an 8843 mRNA (e.g., in a biological sample) or a genetic alteration in an 8843 gene, and to modulate 8843 activity, as described further below. The 8843 proteins can be used to treat disorders characterized by insufficient or excessive production of an 8843 substrate or production of 8843 inhibitors. In addition, the 8843 proteins can be used to screen for naturally occurring 8843 substrates, to screen for drugs or compounds which modulate 8843 activity, as well as to treat disorders characterized by insufficient or excessive production of 8843 protein or production of 8843 protein forms which have decreased, aberrant or unwanted activity compared to 8843 wild type protein (e.g., aberrant, increased or deficient, erythroid proliferation, e.g., an erythroleukemia and, aberrant, increased or deficient erythroid differentiation, e.g., an anemia). Moreover, the anti-8843 antibodies of the invention can be used to detect and isolate 8843 proteins, regulate the bioavailability of 8843 proteins, and modulate 8843 activity.

A method of evaluating a compound for the ability to interact with, e.g., bind, a subject 8843 polypeptide is provided. The method includes: contacting the compound with the subject 8843 polypeptide; and evaluating ability of the compound to interact with, e.g., to bind or form a complex with the subject 8843 polypeptide. This method can be performed in vitro, e.g., in a cell free system, or in vivo, e.g., in a two-hybrid interaction trap assay. This method can be used to identify naturally occurring molecules which interact with subject 8843 polypeptide. It can also be used to find natural or synthetic inhibitors of subject 8843 polypeptide. Screening methods are discussed in more detail below.

Screening Assays:

The invention provides methods (also referred to herein as "screening assays") for identifying modulators, i.e., candidate or test compounds or agents (e.g., proteins, peptides,

peptidomimetics, peptoids, small molecules or other drugs) which bind to 8843 proteins, have a stimulatory or inhibitory effect on, for example, 8843 expression or 8843 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of an 8843 substrate. Compounds thus identified can be used to modulate the activity of target gene products (e.g., 8843 genes) in a therapeutic protocol, to elaborate the biological function of the target gene product, or to identify compounds that disrupt normal target gene interactions.

In one embodiment, the invention provides assays for screening candidate or test compounds, which are substrates of an 8843 protein or polypeptide or a biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds, which bind to or modulate the activity of an 8843 protein or polypeptide or a biologically active portion thereof.

The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive; see, e.g., Zuckermann, R.N. et al. *J. Med. Chem.* 1994, 37: 2678-85); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann et al. (1994). *J. Med. Chem.* 37:2678; Cho et al. (1993) *Science* 261:1303; Carrell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop et al. (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull et al. (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and

Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla et al. (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses an 8843 protein or biologically active portion thereof is contacted with a test compound, and the ability of the test compound to modulate 8843 activity is determined. Determining the ability of the test compound to modulate 8843 activity can be accomplished by monitoring, for example, protein phosphatase activity. The cell, for example, can be of mammalian origin, e.g., human.

The ability of the test compound to modulate 8843 binding to a compound, e.g., an 8843 substrate, or to bind to 8843 can also be evaluated. This can be accomplished, for example, by coupling the compound, e.g., the substrate, with a radioisotope or enzymatic label such that binding of the compound, e.g., the substrate, to 8843 can be determined by detecting the labeled compound, e.g., substrate, in a complex. Alternatively, 8843 could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate 8843 binding to an 8843 substrate in a complex. For example, compounds (e.g., 8843 substrates) can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

The ability of a compound (e.g., an 8843 substrate) to interact with 8843 with or without the labeling of any of the interactants can be evaluated. For example, a microphysiometer can be used to detect the interaction of a compound with 8843 without the labeling of either the compound or the 8843. McConnell, H. M. et al. (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (e.g., Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and 8843.

In yet another embodiment, a cell-free assay is provided in which an 8843 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the 8843 protein or biologically active portion thereof is evaluated. Preferred biologically active portions of the 8843 proteins to be used in assays of the present

invention include fragments, which participate in interactions with non-8843 molecules, e.g., fragments with high surface probability scores.

Soluble and/or membrane-bound forms of isolated proteins (e.g., 8843 proteins or biologically active portions thereof) can be used in the cell-free assays of the invention. When membrane-bound forms of the protein are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

Cell-free assays involve preparing a reaction mixture of the target gene protein and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected.

The interaction between two molecules can also be detected, e.g., using fluorescence energy transfer (FET) (see, for example, Lakowicz *et al.*, U.S. Patent No. 5,631,169; Stavrianopoulos, et al., U.S. Patent No. 4,868,103). A fluorophore label on the first, 'donor' molecule is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second, 'acceptor' molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the 'donor' protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the 'acceptor' molecule label may be differentiated from that of the 'donor'. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (e.g., using a fluorimeter).

In another embodiment, determining the ability of the 8843 protein to bind to a target molecule can be accomplished using real-time Biomolecular Interaction Analysis (BIA) (see, e.g., Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo et al. (1995) *Curr. Opin. Struct. Biol.* 5:699-705). "Surface plasmon resonance" or "BIA" detects biospecific interactions in real time, without labeling any of the interactants (e.g.,

BIAcore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal which can be used as an indication of real-time reactions between biological molecules.

In one embodiment, the target gene product or the test substance is anchored onto a solid phase. The target gene product/test compound complexes anchored on the solid phase can be detected at the end of the reaction. Preferably, the target gene product can be anchored onto a solid surface, and the test compound, (which is not anchored), can be labeled, either directly or indirectly, with detectable labels discussed herein.

It may be desirable to immobilize either 8843, an anti-8843 antibody or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to an 8843 protein, or interaction of an 8843 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/8843 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or 8843 protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of 8843 binding or activity determined using standard techniques.

Other techniques for immobilizing either an 8843 protein or a target molecule on matrices include using conjugation of biotin and streptavidin. Biotinylated 8843 protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical).

In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody).

In one embodiment, this assay is performed utilizing antibodies reactive with 8843 protein or target molecules but which do not interfere with binding of the 8843 protein to its target molecule. Such antibodies can be derivatized to the wells of the plate, and unbound target or 8843 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the 8843 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the 8843 protein or target molecule.

Alternatively, cell free assays can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components, by any of a number of standard techniques, including but not limited to: differential centrifugation (see, for example, Rivas, G., and Minton, A.P., *Trends Biochem Sci* 1993 Aug;18(8):284-7); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (see, e.g., Ausubel, F. et al., eds. Current Protocols in Molecular Biology 1999, J. Wiley: New York.); and immunoprecipitation (see, for example, Ausubel, F. et al., eds. Current Protocols in Molecular Biology 1999, J. Wiley: New York). Such resins and chromatographic techniques are known to one skilled in the art (see, e.g., Heegaard, N.H., *J Mol Recognit* 1998 Winter;11(1-6):141-8; Hage, D.S., and Tweed, S.A. *J Chromatogr B Biomed Sci Appl* 1997 Oct 10;699(1-2):499-525). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

In a preferred embodiment, the assay includes contacting the 8843 protein or biologically active portion thereof with a known compound which binds 8843 to form an

assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an 8843 protein, wherein determining the ability of the test compound to interact with an 8843 protein includes determining the ability of the test compound to preferentially bind to 8843 or biologically active portion thereof, or to modulate the activity of a target molecule, as compared to the known compound.

The target gene products of the invention can, *in vivo*, interact with one or more cellular or extracellular macromolecules, such as proteins. For the purposes of this discussion, such cellular and extracellular macromolecules are referred to herein as "binding partners." Compounds that disrupt such interactions can be useful in regulating the activity of the target gene product. Such compounds can include, but are not limited to molecules such as antibodies, peptides, and small molecules. The preferred target genes/products for use in this embodiment are the 8843 genes herein identified. In an alternative embodiment, the invention provides methods for determining the ability of the test compound to modulate the activity of an 8843 protein through modulation of the activity of a downstream effector of an 8843 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined, or the binding of the effector to an appropriate target can be determined, as previously described.

To identify compounds that interfere with the interaction between the target gene product and its cellular or extracellular binding partner(s), a reaction mixture containing the target gene product and the binding partner is prepared, under conditions and for a time sufficient, to allow the two products to form complex. In order to test an inhibitory agent, the reaction mixture is provided in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be added at a time subsequent to the addition of the target gene and its cellular or extracellular binding partner. Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the target gene product and the cellular or extracellular binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the target gene product and the interactive binding partner.

Additionally, complex formation within reaction mixtures containing the test compound and normal target gene product can also be compared to complex formation within reaction mixtures containing the test compound and mutant target gene product. This

comparison can be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal target gene products.

These assays can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the target gene product or the binding partner onto a solid phase, and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction between the target gene products and the binding partners, e.g., by competition, can be identified by conducting the reaction in the presence of the test substance. Alternatively, test compounds that disrupt preformed complexes, e.g., compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are briefly described below.

In a heterogeneous assay system, either the target gene product or the interactive cellular or extracellular binding partner, is anchored onto a solid surface (e.g., a microtiter plate), while the non-anchored species is labeled, either directly or indirectly. The anchored species can be immobilized by non-covalent or covalent attachments. Alternatively, an immobilized antibody specific for the species to be anchored can be used to anchor the species to the solid surface.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (e.g., by washing) and any complexes formed will remain immobilized on the solid surface. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components,

and complexes detected; e.g., using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. For example, a preformed complex of the target gene product and the interactive cellular or extracellular binding partner product is prepared in that either the target gene products or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, e.g., U.S. Patent No. 4,109,496 that utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt target gene product-binding partner interaction can be identified.

In yet another aspect, the 8843 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos et al. (1993) *Cell* 72:223-232; Madura et al. (1993) *J. Biol. Chem.* 268:12046-12054; Bartel et al. (1993) *Biotechniques* 14:920-924; Iwabuchi et al. (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with 8843 ("8843-binding proteins" or "8843-bp") and are involved in 8843 activity. Such 8843-bps can be activators or inhibitors of signals by the 8843 proteins or 8843 targets as, for example, downstream elements of an 8843-mediated signaling pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for an 8843 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. (Alternatively the: 8843 protein can be the fused to the activator domain.) If the "bait" and the "prey" proteins are able to interact, *in vivo,* forming an 8843-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., lacZ) which is operably linked to a transcriptional regulatory site

responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the 8843 protein.

In another embodiment, modulators of 8843 expression are identified. For example, a cell or cell free mixture is contacted with a candidate compound and the expression of 8843 mRNA or protein evaluated relative to the level of expression of 8843 mRNA or protein in the absence of the candidate compound. When expression of 8843 mRNA or protein is greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of 8843 mRNA or protein expression. Alternatively, when expression of 8843 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of 8843 mRNA or protein expression. The level of 8843 mRNA or protein expression can be determined by methods described herein for detecting 8843 mRNA or protein.

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of an 8843 protein can be confirmed *in vivo*, e.g., in an animal such as an animal model for aberrant (increased or deficient) erythroid proliferation and differentiation.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein (e.g., an 8843 modulating agent, an antisense 8843 nucleic acid molecule, an 8843-specific antibody, or an 8843-binding partner) in an appropriate animal model to determine the efficacy, toxicity, side effects, or mechanism of action, of treatment with such an agent. Furthermore, novel agents identified by the above-described screening assays can be used for treatments as described herein.

Detection Assays

Portions or fragments of the nucleic acid sequences identified herein can be used as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome e.g., to locate gene regions associated with genetic disease or to associate 8843 with a disease; (ii) identify an individual from a minute

biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

Chromosome Mapping

The 8843 nucleotide sequences or portions thereof can be used to map the location of the 8843 genes on a chromosome. This process is called chromosome mapping. Chromosome mapping is useful in correlating the 8843 sequences with genes associated with disease.

Briefly, 8843 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the 8843 nucleotide sequences. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the 8843 sequences will yield an amplified fragment.

A panel of somatic cell hybrids in which each cell line contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, can allow easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. et al. (1983) *Science* 220:919-924).

Other mapping strategies e.g., in situ hybridization (described in Fan, Y. et al. (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries can be used to map 8843 to a chromosomal location.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma et al., Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are

more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. et al. (1987) *Nature,* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the 8843 gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

Tissue Typing

8843 sequences can be used to identify individuals from biological samples using, e.g., restriction fragment length polymorphism (RFLP). In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, the fragments separated, e.g., in a Southern blot, and probed to yield bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can also be used to determine the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the 8843 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it. Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences.

Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 can provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from 8843 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

Use of Partial 8843 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 (e.g., fragments derived from the noncoding regions of SEQ ID NO:1 having a length of at least 20 bases, preferably at least 30 bases) are particularly appropriate for this use.

The 8843 nucleotide sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, e.g., a blood sample, a bone

marrow sample, or a spleen. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such 8843 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, e.g., 8843 primers or probes can be used to screen tissue culture for contamination (i.e. screen for the presence of a mixture of different types of cells in a culture).

Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual.

Generally, the invention provides, a method of determining if a subject is at risk for a disorder related to a lesion in or the misexpression of a gene which encodes 8843.

Such disorders include, e.g., a disorder associated with the misexpression of 8843 gene; a disorder of erythroid cells; a disorder of CD34+ cells.

The method includes one or more of the following:

detecting, in a tissue of the subject, the presence or absence of a mutation which affects the expression of the 8843 gene, or detecting the presence or absence of a mutation in a region which controls the expression of the gene, e.g., a mutation in the 5' control region;

detecting, in a tissue of the subject, the presence or absence of a mutation which alters the structure of the 8843 gene;

detecting, in a tissue of the subject, the misexpression of the 8843 gene, at the mRNA level, e.g., detecting a non-wild type level of a mRNA ;

detecting, in a tissue of the subject, the misexpression of the gene, at the protein level, e.g., detecting a non-wild type level of an 8843 polypeptide.

In preferred embodiments the method includes: ascertaining the existence of at least one of: a deletion of one or more nucleotides from the 8843 gene; an insertion of one or more nucleotides into the gene, a point mutation, e.g., a substitution of one or more nucleotides of the gene, a gross chromosomal rearrangement of the gene, e.g., a translocation, inversion, or deletion.

For example, detecting the genetic lesion can include: (i) providing a probe/primer including an oligonucleotide containing a region of nucleotide sequence which hybridizes to a sense or antisense sequence from SEQ ID NO:1, or naturally occurring mutants thereof or

5' or 3' flanking sequences naturally associated with the 8843 gene; (ii) exposing the probe/primer to nucleic acid of the tissue; and detecting, by hybridization, e.g., *in situ* hybridization, of the probe/primer to the nucleic acid, the presence or absence of the genetic lesion.

In preferred embodiments detecting the misexpression includes ascertaining the existence of at least one of: an alteration in the level of a messenger RNA transcript of the 8843 gene; the presence of a non-wild type splicing pattern of a messenger RNA transcript of the gene; or a non-wild type level of 8843.

Methods of the invention can be used prenatally or to determine if a subject's offspring will be at risk for a disorder.

In preferred embodiments the method includes determining the structure of an 8843 gene, an abnormal structure being indicative of risk for the disorder.

In preferred embodiments the method includes contacting a sample form the subject with an antibody to the 8843 protein or a nucleic acid, which hybridizes specifically with the gene. There and other embodiments are discussed below.

Diagnostic and Prognostic Assays

The presence, level, or absence of 8843 protein or nucleic acid in a biological sample can be evaluated by obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting 8843 protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes 8843 protein such that the presence of 8843 protein or nucleic acid is detected in the biological sample. The term "biological sample" includes tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. A preferred biological sample is serum. The level of expression of the 8843 gene can be measured in a number of ways, including, but not limited to: measuring the mRNA encoded by the 8843 genes; measuring the amount of protein encoded by the 8843 genes; or measuring the activity of the protein encoded by the 8843 genes.

The level of mRNA corresponding to the 8843 gene in a cell can be determined both by *in situ* and by *in vitro* formats.

The isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One preferred diagnostic method for the detection of mRNA levels

involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length 8843 nucleic acid, such as the nucleic acid of SEQ ID NO:1, or the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 8843 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays are described herein.

In one format, mRNA (or cDNA) is immobilized on a surface and contacted with the probes, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative format, the probes are immobilized on a surface and the mRNA (or cDNA) is contacted with the probes, for example, in a two-dimensional gene chip array. A skilled artisan can adapt known mRNA detection methods for use in detecting the level of mRNA encoded by the 8843 genes.

The level of mRNA in a sample that is encoded by one of 8843 can be evaluated with nucleic acid amplification, e.g., by rtPCR (Mullis, 1987, U.S. Patent No. 4,683,202), ligase chain reaction (Barany, 1991, Proc. Natl. Acad. Sci. USA *88*:189-193), self sustained sequence replication (Guatelli et al., 1990, Proc. Natl. Acad. Sci. USA *87*:1874-1878), transcriptional amplification system (Kwoh et al., 1989, Proc. Natl. Acad. Sci. USA *86*:1173-1177), Q-Beta Replicase (Lizardi et al., 1988, Bio/Technology *6*:1197), rolling circle replication (Lizardi et al., U.S. Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques known in the art. As used herein, amplification primers are defined as being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers.

For *in situ* methods, a cell or tissue sample can be prepared/processed and immobilized on a support, typically a glass slide, and then contacted with a probe that can hybridize to mRNA that encodes the 8843 gene being analyzed.

In another embodiment, the methods further contacting a control sample with a compound or agent capable of detecting 8843 mRNA, or genomic DNA, and comparing the presence of 8843 mRNA or genomic DNA in the control sample with the presence of 8843 mRNA or genomic DNA in the test sample.

A variety of methods can be used to determine the level of protein encoded by 8843. In general, these methods include contacting an agent that selectively binds to the protein, such as an antibody with a sample, to evaluate the level of protein in the sample. In a preferred embodiment, the antibody bears a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with a detectable substance. Examples of detectable substances are provided herein.

The detection methods can be used to detect 8843 protein in a biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of 8843 protein include enzyme linked immunosorbent assays (ELISAs), immunoprecipitations, immunofluorescence, enzyme immunoassay (EIA), radioimmunoassay (RIA), and Western blot analysis. *In vivo* techniques for detection of 8843 protein include introducing into a subject a labeled anti-8843 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In another embodiment, the methods further include contacting the control sample with a compound or agent capable of detecting 8843 protein, and comparing the presence of 8843 protein in the control sample with the presence of 8843 protein in the test sample.

The invention also includes kits for detecting the presence of 8843 in a biological sample. For example, the kit can include a compound or agent capable of detecting 8843 protein or mRNA in a biological sample; and a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect 8843 protein or nucleic acid.

For antibody-based kits, the kit can include: (1) a first antibody (e.g., attached to a solid support) which binds to a polypeptide corresponding to a marker of the invention; and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit can include: (1) an oligonucleotide, e.g., a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding a polypeptide corresponding to a marker of the invention or (2) a pair of primers useful for amplifying a nucleic acid molecule corresponding to a marker of the invention. The kit can also includes a buffering agent, a preservative, or a protein stabilizing agent. The kit can also includes components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit.

The diagnostic methods described herein can identify subjects having, or at risk of developing, a disease or disorder associated with misexpressed or aberrant or unwanted 8843 expression or activity. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as pain or deregulated cell proliferation.

In one embodiment, a disease or disorder associated with aberrant or unwanted 8843 expression or activity is identified. A test sample is obtained from a subject and 8843 protein or nucleic acid (e.g., mRNA or genomic DNA) is evaluated, wherein the level, e.g., the presence or absence, of 8843 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted 8843 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest, including a biological fluid (e.g., serum), cell sample, or tissue.

The prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted 8843 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a cell aberrant or deficient erythroid proliferation or differentiation. Preferred disorders include erythroleukemias, anemias, and erythrocytosis.

The methods of the invention can also be used to detect genetic alterations in an 8843 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in 8843 protein activity or nucleic acid expression, such as a aberrant or deficient erythroid proliferation and differentiation. In preferred embodiments,

the methods include detecting, in a sample from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding an 8843-protein, or the mis-expression of the 8843 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from an 8843 gene; 2) an addition of one or more nucleotides to an 8843 gene; 3) a substitution of one or more nucleotides of an 8843 gene, 4) a chromosomal rearrangement of an 8843 gene; 5) an alteration in the level of a messenger RNA transcript of an 8843 gene, 6) aberrant modification of an 8843 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of an 8843 gene, 8) a non-wild type level of an 8843-protein, 9) allelic loss of an 8843 gene, and 10) inappropriate post-translational modification of an 8843-protein.

An alteration can be detected without a probe/primer in a polymerase chain reaction, such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR), the latter of which can be particularly useful for detecting point mutations in the 8843-gene. This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (e.g., genomic, mRNA or both) from the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to an 8843 gene under conditions such that hybridization and amplification of the 8843-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. et al., (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. et al., (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. et al. (1988) *Bio-Technology* 6:1197), or other nucleic acid amplification methods, followed by the detection of the amplified molecules using techniques known to those of skill in the art.

In another embodiment, mutations in an 8843 gene from a sample cell can be identified by detecting alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined, e.g., by gel

electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in 8843 can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, two dimensional arrays, e.g., chip based arrays. Such arrays include a plurality of addresses, each of which is positionally distinguishable from the other. A different probe is located at each address of the plurality. The arrays can have a high density of addresses, e.g., can contain hundreds or thousands of oligonucleotides probes (Cronin, M.T. et al. (1996) *Human Mutation* 7: 244-255; Kozal, M.J. et al. (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in 8843 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra.* Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the 8843 gene and detect mutations by comparing the sequence of the sample 8843 with the corresponding wild-type (control) sequence. Automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry.

Other methods for detecting mutations in the 8843 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers et al. (1985) *Science* 230:1242; Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba et al. (1992) *Methods Enzymol.* 217:286-295).

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in 8843 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli*

cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662; U.S. Patent No. 5,459,039).

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in 8843 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control 8843 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen et al. (1991) *Trends Genet* 7:5).

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230).

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.*

(1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.,* in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an 8843 gene.

Use of 8843 Molecules as Surrogate Markers

The 8843 molecules of the invention are also useful as markers of disorders or disease states, as markers for precursors of disease states, as markers for predisposition of disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, absence and/or quantity of the 8843 molecules of the invention may be detected, and may be correlated with one or more biological states in vivo. For example, the 8843 molecules of the invention may serve as surrogate markers for one or more disorders or disease states or for conditions leading up to disease states. As used herein, a "surrogate marker" is an objective biochemical marker which correlates with the absence or presence of a disease or disorder, or with the progression of a disease or disorder (e.g., with the presence or absence of a tumor). The presence or quantity of such markers is independent of the disease. Therefore, these markers may serve to indicate whether a particular course of treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent of a disease state or disorder is difficult to assess through standard methodologies (e.g., early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is reached (e.g., an assessment of cardiovascular disease may be made using cholesterol levels as a surrogate marker, and an

analysis of HIV infection may be made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of surrogate markers in the art include: Koomen et al. (2000) J. Mass. Spectrom. 35: 258-264; and James (1994) AIDS Treatment News Archive 209.

The 8843 molecules of the invention are also useful as pharmacodynamic markers. As used herein, a "pharmacodynamic marker" is an objective biochemical marker which correlates specifically with drug effects. The presence or quantity of a pharmacodynamic marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence or activity of the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is either expressed or transcribed or not expressed or transcribed in that tissue in relationship to the level of the drug. In this fashion, the distribution or uptake of the drug may be monitored by the pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic marker may be related to the presence or quantity of the metabolic product of a drug, such that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug in vivo. Pharmacodynamic markers are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since even a small amount of a drug may be sufficient to activate multiple rounds of marker (e.g., an 8843 marker) transcription or expression, the amplified marker may be in a quantity which is more readily detectable than the drug itself. Also, the marker may be more easily detected due to the nature of the marker itself; for example, using the methods described herein, anti-8843 antibodies may be employed in an immune-based detection system for an 8843 protein marker, or 8843-specific radiolabeled probes may be used to detect an 8843 mRNA marker. Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the range of possible direct observations. Examples of the use of pharmacodynamic markers in the art include: Matsuda et al. US 6,033,862; Hattis et al. (1991) Env. Health Perspect. 90: 229-238; Schentag (1999) Am. J. Health-Syst. Pharm. 56 Suppl. 3: S21-S24; and Nicolau (1999) Am, J. Health-Syst. Pharm. 56 Suppl. 3: S16-S20.

The 8843 molecules of the invention are also useful as pharmacogenomic markers. As used herein, a "pharmacogenomic marker" is an objective biochemical marker which correlates with a specific clinical drug response or susceptibility in a subject (see, e.g.,

McLeod et al. (1999) Eur. J. Cancer 35(12): 1650-1652). The presence or quantity of the pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success, may be selected. For example, based on the presence or quantity of RNA, or protein (e.g., 8843 protein or RNA) for specific tumor markers in a subject, a drug or course of treatment may be selected that is optimized for the treatment of the specific tumor likely to be present in the subject. Similarly, the presence or absence of a specific sequence mutation in 8843 DNA may correlate 8843 drug response. The use of pharmacogenomic markers therefore permits the application of the most appropriate treatment for each subject without having to administer the therapy.

Pharmaceutical Compositions

The nucleic acid and polypeptides, fragments thereof, as well as anti-8843 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions. Such compositions typically include the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The

parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, e.g., gelatin capsules. Oral

compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The protein or polypeptide can be administered one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to

effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

For antibodies, the preferred dosage is 0.1 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is described by Cruikshank et al. ((1997) J. Acquired Immune Deficiency Syndromes and Human Retrovirology 14:193).

The present invention encompasses agents or compounds which modulate expression or activity. An agent or compound may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. When one or more of these small molecules is to be administered to an animal (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a

physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

An antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, .alpha.-interferon, .beta.-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted 8843 expression or activity. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the 8843 molecules of the present invention or 8843 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted 8843 expression or activity, by administering to the subject an 8843 or an agent which modulates 8843 expression or at

least one 8843 activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted 8843 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the 8843 aberrance, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of 8843 aberrance, for example, an 8843, 8843 agonist or 8843 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

It is possible that some 8843 disorders can be caused, at least in part, by an abnormal level of gene product, or by the presence of a gene product exhibiting abnormal activity. As such, the reduction in the level and/or activity of such gene products would bring about the amelioration of disorder symptoms. Relevant disorders can include erythroid-associated disorders, e.g., anemias, neoplasias of the erythroid lineage or of CD34+ cells, e.g., leukemias, and erythrocytosis, i.e. excessive erythroid differentiation.

As the 8834 molecules are expressed in hepatic cells, kidney, lung, and dermal cells, these molecules can be used diagnostically and therapeutically to treat/diagnose hepatic, kidney, lung, and dermal disorders.

Examples of disorders of the lung include, but are not limited to, congenital anomalies; atelectasis; diseases of vascular origin, such as pulmonary congestion and edema, including hemodynamic pulmonary edema and edema caused by microvascular injury, adult respiratory distress syndrome (diffuse alveolar damage), pulmonary embolism, hemorrhage, and infarction, and pulmonary hypertension and vascular sclerosis; chronic obstructive pulmonary disease, such as emphysema, chronic bronchitis, bronchial asthma, and bronchiectasis; diffuse interstitial (infiltrative, restrictive) diseases, such as pneumoconioses, sarcoidosis, idiopathic pulmonary fibrosis, desquamative interstitial pneumonitis, hypersensitivity pneumonitis, pulmonary eosinophilia (pulmonary infiltration with eosinophilia), *Bronchiolitis obliterans*-organizing pneumonia, diffuse pulmonary hemorrhage syndromes, including Goodpasture syndrome, idiopathic pulmonary hemosiderosis and other hemorrhagic syndromes, pulmonary involvement in collagen vascular disorders, and pulmonary alveolar proteinosis; complications of therapies, such as drug-induced lung disease, radiation-induced lung disease, and lung transplantation; tumors, such as bronchogenic carcinoma, including paraneoplastic syndromes, bronchioloalveolar carcinoma, neuroendocrine tumors, such as bronchial carcinoid, miscellaneous tumors, and

metastatic tumors; pathologies of the pleura, including inflammatory pleural effusions, noninflammatory pleural effusions, pneumothorax, and pleural tumors, including solitary fibrous tumors (pleural fibroma) and malignant mesothelioma.

Disorders involving the liver include, but are not limited to, hepatic injury; jaundice and cholestasis, such as bilirubin and bile formation; hepatic failure and cirrhosis, such as cirrhosis, portal hypertension, including ascites, portosystemic shunts, and splenomegaly; infectious disorders, such as viral hepatitis, including hepatitis A-E infection and infection by other hepatitis viruses, clinicopathologic syndromes, such as the carrier state, asymptomatic infection, acute viral hepatitis, chronic viral hepatitis, and fulminant hepatitis; autoimmune hepatitis; drug- and toxin-induced liver disease, such as alcoholic liver disease; inborn errors of metabolism and pediatric liver disease, such as hemochromatosis, Wilson disease, a_1-antitrypsin deficiency, and neonatal hepatitis; intrahepatic biliary tract disease, such as secondary biliary cirrhosis, primary biliary cirrhosis, primary sclerosing cholangitis, and anomalies of the biliary tree; circulatory disorders, such as impaired blood flow into the liver, including hepatic artery compromise and portal vein obstruction and thrombosis, impaired blood flow through the liver, including passive congestion and centrilobular necrosis and peliosis hepatis, hepatic vein outflow obstruction, including hepatic vein thrombosis (Budd-Chiari syndrome) and veno-occlusive disease; hepatic disease associated with pregnancy, such as preeclampsia and eclampsia, acute fatty liver of pregnancy, and intrehepatic cholestasis of pregnancy; hepatic complications of organ or bone marrow transplantation, such as drug toxicity after bone marrow transplantation, graft-versus-host disease and liver rejection, and nonimmunologic damage to liver allografts; tumors and tumorous conditions, such as nodular hyperplasias, adenomas, and malignant tumors, including primary carcinoma of the liver and metastatic tumors.

Disorders involving the kidney include, but are not limited to, congenital anomalies including, but not limited to, cystic diseases of the kidney, that include but are not limited to, cystic renal dysplasia, autosomal dominant (adult) polycystic kidney disease, autosomal recessive (childhood) polycystic kidney disease, and cystic diseases of renal medulla, which include, but are not limited to, medullary sponge kidney, and nephronophthisis-uremic medullary cystic disease complex, acquired (dialysis-associated) cystic disease, such as simple cysts; glomerular diseases including pathologies of glomerular injury that include, but are not limited to, in situ immune complex deposition, that includes, but is not limited to, anti-GBM nephritis, Heymann nephritis, and antibodies against planted antigens, circulating immune

complex nephritis, antibodies to glomerular cells, cell-mediated immunity in glomerulonephritis, activation of alternative complement pathway, epithelial cell injury, and pathologies involving mediators of glomerular injury including cellular and soluble mediators, acute glomerulonephritis, such as acute proliferative (poststreptococcal, postinfectious) glomerulonephritis, including but not limited to, poststreptococcal glomerulonephritis and nonstreptococcal acute glomerulonephritis, rapidly progressive (crescentic) glomerulonephritis, nephrotic syndrome, membranous glomerulonephritis (membranous nephropathy), minimal change disease (lipoid nephrosis), focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, IgA nephropathy (Berger disease), focal proliferative and necrotizing glomerulonephritis (focal glomerulonephritis), hereditary nephritis, including but not limited to, Alport syndrome and thin membrane disease (benign familial hematuria), chronic glomerulonephritis, glomerular lesions associated with systemic disease, including but not limited to, systemic lupus erythematosus, Henoch-Schönlein purpura, bacterial endocarditis, diabetic glomerulosclerosis, amyloidosis, fibrillary and immunotactoid glomerulonephritis, and other systemic disorders; diseases affecting tubules and interstitium, including acute tubular necrosis and tubulointerstitial nephritis, including but not limited to, pyelonephritis and urinary tract infection, acute pyelonephritis, chronic pyelonephritis and reflux nephropathy, and tubulointerstitial nephritis induced by drugs and toxins, including but not limited to, acute drug-induced interstitial nephritis, analgesic abuse nephropathy, nephropathy associated with nonsteroidal anti-inflammatory drugs, and other tubulointerstitial diseases including, but not limited to, urate nephropathy, hypercalcemia and nephrocalcinosis, and multiple myeloma; diseases of blood vessels including benign nephrosclerosis, malignant hypertension and accelerated nephrosclerosis, renal artery stenosis, and thrombotic microangiopathies including, but not limited to, classic (childhood) hemolytic-uremic syndrome, adult hemolytic-uremic syndrome/thrombotic thrombocytopenic purpura, idiopathic HUS/TTP, and other vascular disorders including, but not limited to, atherosclerotic ischemic renal disease, atheroembolic renal disease, sickle cell disease nephropathy, diffuse cortical necrosis, and renal infarcts; urinary tract obstruction (obstructive uropathy); urolithiasis (renal calculi, stones); and tumors of the kidney including, but not limited to, benign tumors, such as renal papillary adenoma, renal fibroma or hamartoma (renomedullary interstitial cell tumor), angiomyolipoma, and oncocytoma, and malignant tumors, including renal cell carcinoma (hypernephroma, adenocarcinoma of kidney), which includes urothelial carcinomas of renal pelvis.

Examples of disorders of the skin include but are not limited to, disorders of pigmentation and melanocytes, including but not limited to, vitiligo, freckle, melasma, lentigo, nevocellular nevus, dysplastic nevi, and malignant melanoma; benign epithelial tumors, including but not limited to, seborrheic keratoses, acanthosis nigricans, fibroepithelial polyp, epithelial cyst, keratoacanthoma, and adnexal (appendage) tumors; premalignant and malignant epidermal tumors, including but not limited to, actinic keratosis, squamous cell carcinoma, basal cell carcinoma, and merkel cell carcinoma; tumors of the dermis, including but not limited to, benign fibrous histiocytoma, dermatofibrosarcoma protuberans, xanthomas, and dermal vascular tumors; tumors of cellular immigrants to the skin, including but not limited to, histiocytosis X, mycosis fungoides (cutaneous T-cell lymphoma), and mastocytosis; disorders of epidermal maturation, including but not limited to, ichthyosis; acute inflammatory dermatoses, including but not limited to, urticaria, acute eczematous dermatitis, and erythema multiforme; chronic inflammatory dermatoses, including but not limited to, psoriasis, lichen planus, and lupus erythematosus; blistering (bullous) diseases, including but not limited to, pemphigus, bullous pemphigoid, dermatitis herpetiformis, and noninflammatory blistering diseases: epidermolysis bullosa and porphyria; disorders of epidermal appendages, including but not limited to, acne vulgaris; panniculitis, including but not limited to, erythema nodosum and erythema induratum; and infection and infestation, such as verrucae, molluscum contagiosum, impetigo, superficial fungal infections, and arthropod bites, stings, and infestations.

As discussed, successful treatment of 8843 disorders can be brought about by techniques that serve to inhibit the expression or activity of target gene products. For example, compounds, e.g., an agent identified using an assays described above, that proves to exhibit negative modulatory activity, can be used in accordance with the invention to prevent and/or ameliorate symptoms of 8843 disorders. Such molecules can include, but are not limited to peptides, phosphopeptides, small organic or inorganic molecules, or antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and Fab, $F(ab')_2$ and Fab expression library fragments, scFV molecules, and epitope-binding fragments thereof).

Further, antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity. Still further, triple

helix molecules can be utilized in reducing the level of target gene activity. Antisense, ribozyme and triple helix molecules are discussed above.

It is possible that the use of antisense, ribozyme, and/or triple helix molecules to reduce or inhibit mutant gene expression can also reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles, such that the concentration of normal target gene product present can be lower than is necessary for a normal phenotype. In such cases, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity can be introduced into cells via gene therapy method. Alternatively, in instances in that the target gene encodes an extracellular protein, it can be preferable to co-administer normal target gene protein into the cell or tissue in order to maintain the requisite level of cellular or tissue target gene activity.

Another method by which nucleic acid molecules may be utilized in treating or preventing a disease characterized by 8843 expression is through the use of aptamer molecules specific for 8843 protein. Aptamers are nucleic acid molecules having a tertiary structure which permits them to specifically bind to protein ligands (see, e.g., Osborne, et al. *Curr. Opin. Chem Biol.* 1997, 1(1): 5-9; and Patel, D.J. *Curr Opin Chem Biol* 1997 Jun;1(1):32-46). Since nucleic acid molecules may in many cases be more conveniently introduced into target cells than therapeutic protein molecules may be, aptamers offer a method by which 8843 protein activity may be specifically decreased without the introduction of drugs or other molecules which may have pluripotent effects.

Antibodies can be generated that are both specific for target gene product and that reduce target gene product activity. Such antibodies may, therefore, by administered in instances whereby negative modulatory techniques are appropriate for the treatment of 8843 disorders. For a description of antibodies, see the Antibody section above.

In circumstances wherein injection of an animal or a human subject with an 8843 protein or epitope for stimulating antibody production is harmful to the subject, it is possible to generate an immune response against 8843 through the use of anti-idiotypic antibodies (see, for example, Herlyn, D. *Ann Med* 1999;31(1):66-78; and Bhattacharya-Chatterjee, M., and Foon, K.A. *Cancer Treat Res* 1998;94:51-68). If an anti-idiotypic antibody is introduced into a mammal or human subject, it should stimulate the production of anti-anti-idiotypic antibodies, which should be specific to the 8843 protein. Vaccines directed to a disease characterized by 8843 expression may also be generated in this fashion.

In instances where the target antigen is intracellular and whole antibodies are used, internalizing antibodies may be preferred. Lipofectin or liposomes can be used to deliver the antibody or a fragment of the Fab region that binds to the target antigen into cells. Where fragments of the antibody are used, the smallest inhibitory fragment that binds to the target antigen is preferred. For example, peptides having an amino acid sequence corresponding to the Fv region of the antibody can be used. Alternatively, single chain neutralizing antibodies that bind to intracellular target antigens can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population (see e.g., Marasco et al. (1993, Proc. Natl. Acad. Sci. USA 90:7889-7893).

The identified compounds that inhibit target gene expression, synthesis and/or activity can be administered to a patient at therapeutically effective doses to prevent, treat or ameliorate 8843 disorders. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of the disorders.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such

information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

Another example of determination of effective dose for an individual is the ability to directly assay levels of "free" and "bound" compound in the serum of the test subject. Such assays may utilize antibody mimics and/or "biosensors" that have been created through molecular imprinting techniques. The compound which is able to modulate 8843 activity is used as a template, or "imprinting molecule", to spatially organize polymerizable monomers prior to their polymerization with catalytic reagents. The subsequent removal of the imprinted molecule leaves a polymer matrix which contains a repeated "negative image" of the compound and is able to selectively rebind the molecule under biological assay conditions. A detailed review of this technique can be seen in Ansell, R. J. *et al* (1996) *Current Opinion in Biotechnology* **7**:89-94 and in Shea, K.J. (1994) *Trends in Polymer Science* **2**:166-173. Such "imprinted" affinity matrixes are amenable to ligand-binding assays, whereby the immobilized monoclonal antibody component is replaced by an appropriately imprinted matrix. An example of the use of such matrixes in this way can be seen in Vlatakis, G. *et al* (1993) *Nature* 361:645-647. Through the use of isotope-labeling, the "free" concentration of compound which modulates the expression or activity of 8843 can be readily monitored and used in calculations of IC_{50}.

Such "imprinted" affinity matrixes can also be designed to include fluorescent groups whose photon-emitting properties measurably change upon local and selective binding of target compound. These changes can be readily assayed in real time using appropriate fiberoptic devices, in turn allowing the dose in a test subject to be quickly optimized based on its individual IC_{50}. An rudimentary example of such a "biosensor" is discussed in Kriz, D. *et al* (1995) *Analytical Chemistry* 67:2142-2144.

Another aspect of the invention pertains to methods of modulating 8843 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with an 8843, agent or compound that modulates one or more of the activities of 8843 protein activity associated with the cell. An agent that modulates 8843 protein activity can be an agent or a compound as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of an 8843 protein (e.g., an 8843 substrate or receptor), an 8843 antibody, an 8843 agonist or antagonist, a peptidomimetic of an 8843 agonist or antagonist, or other small molecule. The terms "agent" and "compound" are used interchangeably herein.

In one embodiment, the agent stimulates one or 8843 activities. Examples of such stimulatory agents include active 8843 protein and a nucleic acid molecule encoding 8843. In another embodiment, the agent inhibits one or more 8843 activities. Examples of such inhibitory agents include antisense 8843 nucleic acid molecules, anti8843 antibodies, and 8843 inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of an 8843 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., up regulates or down regulates) 8843 expression or activity. In another embodiment, the method involves administering an 8843 protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 8843 expression or activity.

Stimulation of 8843 activity is desirable in situations in which 8843 is abnormally downregulated and/or in which increased 8843 activity is likely to have a beneficial effect. For example, stimulation of 8843 activity is desirable in situations in which an 8843 is downregulated and/or in which increased 8843 activity is likely to have a beneficial effect. Likewise, inhibition of 8843 activity is desirable in situations in which 8843 is abnormally upregulated and/or in which decreased 8843 activity is likely to have a beneficial effect.

Pharmacogenomics

The 8843 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on 8843 activity (e.g., 8843 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) 8843 associated disorders (e.g., aberrant or deficient erythroid proliferation and differentiation) associated with aberrant or unwanted 8843 activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining

whether to administer an 8843 molecule or 8843 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with an 8843 molecule or 8843 modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. et al. (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11) :983-985 and Linder, M.W. et al. (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a

drug's target is known (e.g., an 8843 protein of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., an 8843 molecule or 8843 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an 8843 molecule or 8843 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

The present invention further provides methods for identifying new agents, or combinations, that are based on identifying agents that modulate the activity of one or more of the gene products encoded by one or more of the 8843 genes of the present invention, wherein these products may be associated with resistance of the cells to a therapeutic agent. Specifically, the activity of the proteins encoded by the 8843 genes of the present invention can be used as a basis for identifying agents for overcoming agent resistance. By blocking the activity of one or more of the resistance proteins, target cells, e.g., human cells, will become sensitive to treatment with an agent that the unmodified target cells were resistant to.

Monitoring the influence of agents (e.g., drugs) on the expression or activity of an 8843 protein can be applied in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase 8843 gene expression, protein levels, or upregulate 8843 activity, can be monitored in clinical trials of subjects exhibiting decreased 8843 gene expression, protein levels, or downregulated 8843 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease 8843 gene expression, protein levels, or downregulate 8843 activity, can be monitored in clinical trials of subjects exhibiting increased 8843 gene expression, protein levels, or upregulated 8843 activity. In such clinical trials, the expression or activity of an 8843 gene,

and preferably, other genes that have been implicated in, for example, an 8843-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

Other Embodiments

In another aspect, the invention features, a method of analyzing a plurality of capture probes. The method can be used, e.g., to analyze gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., a nucleic acid or peptide sequence; contacting the array with an 8843, preferably purified, nucleic acid, preferably purified, polypeptide, preferably purified, or antibody, and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the 8843 nucleic acid, polypeptide, or antibody.

The capture probes can be a set of nucleic acids from a selected sample, e.g., a sample of nucleic acids derived from a control or non-stimulated tissue or cell.

The method can include contacting the 8843 nucleic acid, polypeptide, or antibody with a first array having a plurality of capture probes and a second array having a different plurality of capture probes. The results of each hybridization can be compared, e.g., to analyze differences in expression between a first and second sample. The first plurality of capture probes can be from a control sample, e.g., a wild type, normal, or non-diseased, non-stimulated, sample, e.g., a biological fluid, tissue, or cell sample. The second plurality of capture probes can be from an experimental sample, e.g., a mutant type, at risk, disease-state or disorder-state, or stimulated, sample, e.g., a biological fluid, tissue, or cell sample.

The plurality of capture probes can be a plurality of nucleic acid probes each of which specifically hybridizes, with an allele of 8843. Such methods can be used to diagnose a subject, e.g., to evaluate risk for a disease or disorder, to evaluate suitability of a selected treatment for a subject, to evaluate whether a subject has a disease or disorder. 8843 is associated with aberrant or deficient erythroid proliferation and differentiation, thus it is useful for evaluating the same.

The method can be used to detect SNPs, as described above.

In another aspect, the invention features, a method of analyzing a plurality of probes. The method is useful, e.g., for analyzing gene expression. The method includes: providing

a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which express 8843 or from a cell or subject in which an 8843 mediated response has been elicited, e.g., by contact of the cell with 8843 nucleic acid or protein, or administration to the cell or subject 8843 nucleic acid or protein; providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which does not express 8843 (or does not express as highly as in the case of the 8843 positive plurality of capture probes) or from a cell or subject which in which an 8843 mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); contacting the array with one or more inquiry probes (which is preferably other than an 8843 nucleic acid, polypeptide, or antibody), and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody.

In another aspect, the invention features, a method of analyzing a plurality of probes or a sample. The method is useful, e.g., for analyzing gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, contacting the array with a first sample from a cell or subject which express or mis express 8843 or from a cell or subject in which an 8843 mediated response has been elicited, e.g., by contact of the cell with 8843 nucleic acid or protein, or administration to the cell or subject 8843 nucleic acid or protein; providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, and contacting the array with a second sample from a cell or subject which does not express 8843 (or does not express as highly as in the case of the 8843 positive plurality of capture probes) or from a cell or subject which in which an 8843 mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); and comparing the binding of the first sample with the binding of the second sample. Binding, e.g., in the case of a nucleic acid, hybridization with a capture

probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody. The same array can be used for both samples or different arrays can be used. If different arrays are used the plurality of addresses with capture probes should be present on both arrays.

In another aspect, the invention features, a method of analyzing 8843, e.g., analyzing structure, function, or relatedness to other nucleic acid or amino acid sequences. The method includes: providing an 8843 nucleic acid or amino acid sequence; comparing the 8843 sequence with one or more preferably a plurality of sequences from a collection of sequences, e.g., a nucleic acid or protein sequence database; to thereby analyze 8843.

The method can include evaluating the sequence identity between an 8843 sequence and a database sequence. The method can be performed by accessing the database at a second site, e.g., over the internet.

In another aspect, the invention features, a set of oligonucleotides, useful, e.g., for identifying SNP's, or identifying specific alleles of 8843. The set includes a plurality of oligonucleotides, each of which has a different nucleotide at an interrogation position, e.g., an SNP or the site of a mutation. In a preferred embodiment, the oligonucleotides of the plurality identical in sequence with one another (except for differences in length). The oligonucleotides can be provided with differential labels, such that an oligonucleotides which hybridizes to one allele provides a signal that is distinguishable from an oligonucleotides which hybridizes to a second allele.

The sequence of an 8843 molecules is provided in a variety of mediums to facilitate use thereof. A sequence can be provided as a manufacture, other than an isolated nucleic acid or amino acid molecule, which contains an 8843. Such a manufacture can provide a nucleotide or amino acid sequence, e.g., an open reading frame, in a form which allows examination of the manufacture using means not directly applicable to examining the nucleotide or amino acid sequences, or a subset thereof, as they exists in nature or in purified form.

A 8843 nucleotide or amino acid sequence can be recorded on computer readable media. As used herein, "computer readable media" refers to any medium that can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media.

A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide or amino acid sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. The skilled artisan can readily adapt any number of data processor structuring formats (*e.g.*, text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

By providing the nucleotide or amino acid sequences of the invention in computer readable form, the skilled artisan can routinely access the sequence information for a variety of purposes. For example, one skilled in the art can use the nucleotide or amino acid sequences of the invention in computer readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. A search is used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif.

As used herein, a "target sequence" can be any DNA or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. Typical sequence lengths of a target sequence are from about 10 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that commercially important fragments, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium for analysis and comparison to other sequences. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention. Examples of such software include, but are not limited to, MacPattern (EMBL), BLASTN and BLASTX (NCBI).

Thus, the invention features a method of making a computer readable record of a sequence of an 8843 sequence which includes recording the sequence on a computer readable matrix. In a preferred embodiment the record includes one or more of the following: identification of an ORF; identification of a domain, region, or site; identification of the start of transcription; identification of the transcription terminator; the full length amino acid sequence of the protein, or a mature form thereof; the 5' end of the translated region.

In another aspect, the invention features, a method of analyzing a sequence. The method includes: providing an 8843 sequence, or record, in computer readable form; comparing a second sequence to the 8843 sequence; thereby analyzing a sequence. Comparison can include comparing to sequences for sequence identity or determining if one sequence is included within the other, e.g., determining if the 8843 sequence includes a sequence being compared. In a preferred embodiment the 8843 or second sequence is stored on a first computer, e.g., at a first site and the comparison is performed, read, or recorded on a second computer, e.g., at a second site. E.g., the 8843 or second sequence can be stored in a public or proprietary database in one computer, and the results of the comparison performed, read, or recorded on a second computer. In a preferred embodiment the record includes one or more of the following: identification of an ORF; identification of a domain, region, or site; identification of the start of transcription; identification of the transcription terminator; the full length amino acid sequence of the protein, or a mature form thereof; the 5' end of the translated region.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

EXAMPLES

Example 1: Identification and Characterization of Human 8843 cDNA

The human 8843 sequence (Fig. 1; SEQ ID NO:1), which is approximately 839 nucleotides long, including untranslated regions, contains a predicted methionine-initiated coding sequence of about 606 nucleotides, including the termination codon (nucleotides indicated as "coding" of SEQ ID NO:1 in Fig. 1; SEQ ID NO:3). The coding sequence encodes a 201 amino acid protein (SEQ ID NO:2).

Example 2: Tissue Distribution of 8843 mRNA

Endogenous human 8834 gene expression was determined using the Perkin-Elmer/ABI 7700 Sequence Detection System which employs TaqMan technology. Briefly, TaqMan technology relies on standard RT-PCR with the addition of a third gene-specific oligonucleotide (referred to as a probe) which has a fluorescent dye coupled to its 5' end (typically 6-FAM) and a quenching dye at the 3' end (typically TAMRA). When the fluorescently tagged oligonucleotide is intact, the fluorescent signal from the 5' dye is quenched. As PCR proceeds, the 5' to 3' nucleolytic activity of Taq polymerase digests the labeled primer, producing a free nucleotide labeled with 6-FAM, which is now detected as a fluorescent signal. The PCR cycle where fluorescence is first released and detected is directly proportional to the starting amount of the gene of interest in the test sample, thus providing a way of quantitating the initial template concentration. Samples can be internally controlled by the addition of a second set of primers/probe specific for a housekeeping gene such as GAPDH which has been labeled with a different fluorophore on the 5' end (typically VIC).

To determine the level of 8834 in various human tissues a primer/probe set was designed using Primer Express (Perkin-Elmer) software and primary cDNA sequence information. Total RNA was prepared from a series of human tissues using an RNeasy kit from Qiagen. First strand cDNA was prepared from 1 μg total RNA using an oligo-dT primer and Superscript II reverse transcriptase (Gibco/BRL). cDNA obtained from approximately 50 ng total RNA was used per TaqMan reaction.

8834 mRNA levels were analyzed in a variety of samples of isolated and/or treated blood cells. High relative expression levels of 8834 mRNA, e.g., greater than 40 units, were observed for megakaryocytes, mast cells, blast forming units (BFU), especially BFUs treated with erythropoietin (Fig. 5). Moderate relative expression levels of 8834 mRNA, between 10 and 40 units, were observed for multiple erythroid samples, and a subset of neutrophil samples (Fig. 5)

High relative expression levels of 8834 mRNA, e.g., greater than 40 units, were observed for mobilized CD34+ peripheral blood cells (mBM), normal bone marrow CD34+ cells, as well as for glycophorin A (low levels) bone marrow cells (>60 units), which are erythroid progenitors (Fig. 6). Moderate relative expression levels of 8834 mRNA, between

10 and 40 units, were observed for CD34+ cord blood cells, CD34+ fetal liver cells, and mobilized CD34+ bone marrow cells (Fig. 6).

8834 mRNA expression levels were also monitored in other hematopoietic lineages and tissues. K582 cells, an erythroid/megakaryocyte cell line, and Hep3b cells had high 8834 expression levels relative to controls (Fig. 7).

Northern blot hybridizations with various RNA samples can be performed under standard conditions and washed under stringent conditions, i.e., 0.2xSSC at 65°C. A DNA probe corresponding to all or a portion of the 8843 cDNA (SEQ ID NO:1) can be used. The DNA was radioactively labeled with ^{32}P-dCTP using the Prime-It Kit (Stratagene, La Jolla, CA) according to the instructions of the supplier. Filters containing mRNA from mouse hematopoietic and endocrine tissues, and cancer cell lines (Clontech, Palo Alto, CA) can be probed in ExpressHyb hybridization solution (Clontech) and washed at high stringency according to manufacturer's recommendations.

Example 3: Recombinant Expression of 8843 in Bacterial Cells

In this example, 8843 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, 8843 is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-8843 fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

Example 4: Expression of Recombinant 8843 Protein in COS Cells

To express the 8843 gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire 8843 protein and an HA tag (Wilson et al. (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the 8843 DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the 8843 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the 8843 coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the 8843_gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5α, SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the 8843-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the 8843 polypeptide is detected by radiolabelling (^{35}S-methionine or ^{35}S-cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labeled for 8 hours with ^{35}S-methionine (or ^{35}S-cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the 8843 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the 8843 polypeptide is detected by radiolabelling and immunoprecipitation using an 8843 specific monoclonal antibody.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. A method for identifying an agent which modulates the activity of an 8834 polypeptide, comprising:

a) providing an 8843 polypeptide selected from the group consisting of:

i) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO: 1, or SEQ ID NO:3, the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, or a complement thereof.

ii) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO: 1, SEQ ID NO:3, or a complement thereof under stringent conditions; and

iii) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2;

b) contacting the 8843 polypeptide, or a cell expressing the polypeptide with a test compound; and

c) determining the effect of the test agent on the activity of the 8843 polypeptide.

2. The method of claim 1, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2.

3. The method of claim 1, wherein the polypeptide comprises a fragment having the amino acid sequence of SEQ ID NO:2 wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2.

4. The method of claim 1, wherein the polypeptide further comprises a heterologous amino acid sequence.

5. The method of claim 1, wherein the activity of the 8834 polypeptide is proliferation, differentiation, or survival of a CD34 positive cell.

6. A method of modulating erythropoiesis, comprising contacting an erythroid cell with an agent that modulates the activity or expression of an 8834 polypeptide, thereby modulating the differentiation of the erythroid cell, wherein the 8834 polypeptide is selected from the group consisting of:

i) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO: 1, or SEQ ID NO:3, the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, or a complement thereof.

ii) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO: 1, SEQ ID NO:3, or a complement thereof under stringent conditions; and

iii) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2;

b) contacting the 8843 polypeptide, or a cell expressing the polypeptide with a test agent; and

c) determining whether the test agent modulates proliferation, differentiation or survival of an erythroid cell.

7. The method of claim 6, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2.

8. The method of claim 6, wherein the erythroid cell is an erythroid progenitor or differentiated cell.

9. The method of claim 6, wherein the agent and the 8834 polypeptide are contacted *in vitro* or *ex vivo*.

10. The method of claim 6, wherein the contacting step is effected *in vivo* in a subject.

11. The method of claim 10, wherein the subject is a human.

12. The method of claim 11, wherein the subject is a patient having an erythroid-associated disorder.

13. The method of claim 6, further comprising the step of contacting of the erythroid cell with a protein selected from the group consisting of G-CSF, GM-CSF, stem cell factor, Flt-3 ligand, IL-3, IL-4, thrombopoietin, and erythropoietin.

14. The method of claim 13, wherein the protein is erythropoietin.

15. A method of modulating erythropoiesis, comprising contacting an erythroid cell with an agent that modulates the activity or expression of an 8834 nucleic acid, thereby modulating the proliferation, differentiation or survival of the erythroid cell, wherein the 8834 nucleic acid is selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______;

b) a nucleic acid molecule comprising a fragment of at least 300 nucleotides of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO:3, or the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______;

c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______;

d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO: 2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______; and

e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO: 1, 3, or a complement thereof, under stringent conditions.

16. A method of treating or preventing an erythroid-associated disorder, in a subject, comprising administering to the subject an effective amount of an agent that modulates the

activity or expression of an 8834 polypeptide such that the erythroid-associated disorder is ameliorated or prevented, wherein the 8834 polypeptide is selected from the group consisting of:

i) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO: 1, or SEQ ID NO:3, the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, or a complement thereof.

ii) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO: 1, SEQ ID NO:3, or a complement thereof under stringent conditions; and

iii) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2.

17. A method of treating or preventing an erythroid-associated disorder, in a subject, comprising administering to the subject an effective amount of an agent that modulates the activity or expression of an 8834 polypeptide such that the erythroid-associated disorder is ameliorated or prevented, wherein the 8834 polypeptide is selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______;

b) a nucleic acid molecule comprising a fragment of at least 300 nucleotides of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO:3, or the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______;

c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______;

d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO: 2, or the

amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______; and

e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO: 1, 3, or a complement thereof, under stringent conditions.

18. A method of modulating the proliferation, differentiation or survival of a CD34 positive cell, comprising contacting the cell with an agent that modulates the activity or expression of an 8834 polypeptide, in an amount effective to modulate the proliferation, differentiation or survival of the cell, wherein the 8834 polypeptide is selected from the group consisting of:

i) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO: 1, or SEQ ID NO:3, the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, or a complement thereof.

ii) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO: 1, SEQ ID NO:3, or a complement thereof under stringent conditions; and

iii) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2.

19. The method of claim 18, wherein the CD34 positive cell is an erythroid cell.

20. The method of claim 18, wherein the CD34 positive cell is a bone marrow cell.

21. The method of claim 18, wherein the CD34 positive cell is positive for glycophorin A protein.

22. The method of claim 18, wherein the agent is selected from the group consisting of a peptide, a phosphopeptide, a small molecule, and an antibody.

23. The method of claim 18, wherein the agent is administered in combination with a cytotoxic agent.

24. A method of modulating the proliferation, differentiation or survival of a CD34 positive cell, comprising contacting the cell with an agent that modulates the activity or expression of an 8834 nucleic acid, in an amount effective to modulate the proliferation, differentiation or survival of the cell, wherein the 8834 nucleic acid is selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______;

b) a nucleic acid molecule comprising a fragment of at least 300 nucleotides of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO:3, or the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______;

c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______;

d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO: 2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______; and

e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO: 1, 3, or a complement thereof, under stringent conditions.

25. A method for evaluating the efficacy of a treatment of an erythroid-associated disorder, in a subject, comprising:

treating a subject with a protocol under evaluation;

assessing the expression level of a 8834 nucleic acid or 8834 polypeptide,

wherein a change in the expression level of 8834 nucleic acid or 8834 polypeptide after the treatment, relative to the level before the treatment, is indicative of the efficacy of the treatment of the erythroid-associated disorder.

26. The method of claim 25 further comprising treating the subject with erythropoietin prior to assessing expression levels.

27. The method of claim 25, wherein the subject is a patient having an erythroid-associated disorder.

28. The method of claim 27, wherein the erythroid disorder is an anemia.

29. The method of claim 27, wherein the erythroid disorder is a leukemia.

30. A method of diagnosing an erythroid-associated disorder, in a subject, comprising:

evaluating the expression or activity of a 8834 nucleic acid or a 8834 polypeptide, such that, a difference in the level of 8834 nucleic acid or 8834 polypeptide relative to a normal subject or a cohort of normal subjects is indicative of the erythroid-associated disorder.

31. An isolated nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3 or the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______;

b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______; and

d) a nucleic acid molecule which encodes a naturally-occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO: 1, SEQ ID NO:3 or a complement thereof, under stringent conditions.

32. An isolated polypeptide selected from the group consisting of:

a) a polypeptide which is encoded by a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO:3, the amino acid sequence encoded by

the cDNA insert of the plasmid deposited with the ATCC as Accession Number _______, or a complement thereof; and

b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO: 1, SEQ ID NO:3, or a complement thereof under stringent conditions.

8843, A NOVEL HUMAN DUAL SPECIFICITY PHOSPHATASE FAMILY MEMBER AND USES THEREOF

Abstract

The invention provides isolated nucleic acids molecules, designated 8843 nucleic acid molecules, which encode novel dual specificity phosphatase members. The invention also provides methods of modulating the differentiation and proliferation of erythroid progenitors, and CD34 positive cells. The invention further provides methods of treating, preventing and diagnosing eythroid-associated disorders such as anemias, leukemias, and erythrocytosis.

START SEQ ID NO: 1 START SEQ ID NO: 3

```
cgcgagcgcg ggggccgacg ggtcgccgct gcgccgggcc ggg atg gcg gcc acc     55
                                                 Met Ala Ala Thr
                                                 START SEQ ID NO: 2

gcg ctg ctg gag gcc ggc ctg gcg cgg gtg ctc ttc tac ccg acg ctg    103
Ala Leu Leu Glu Ala Gly Leu Ala Arg Val Leu Phe Tyr Pro Thr Leu

ctc tac acc ctg ttc cgc ggg aag gtg ccg ggt cgg gcg cac cgg gac    151
Leu Tyr Thr Leu Phe Arg Gly Lys Val Pro Gly Arg Ala His Arg Asp

tgg tac cac cgc atc gac ccc acc gtg ctg ctg ggc gcg ctg ccg ttg    199
Trp Tyr His Arg Ile Asp Pro Thr Val Leu Leu Gly Ala Leu Pro Leu

cgg agc ttg acg cgc cag ctg gta cag gac gag aac gtg cgc ggg gtg    247
Arg Ser Leu Thr Arg Gln Leu Val Gln Asp Glu Asn Val Arg Gly Val

atc acc atg aac gag gag tac gag acg agg ttc ctg tgc aac tct tca    295
Ile Thr Met Asn Glu Glu Tyr Glu Thr Arg Phe Leu Cys Asn Ser Ser

cag gag tgg aag aga cta gga gtc gag cag ctg cgg ctc agc aca gta    343
Gln Glu Trp Lys Arg Leu Gly Val Glu Gln Leu Arg Leu Ser Thr Val

gac atg act ggg atc ccc acc ttg gac aac ctc cag aag gga gtc caa    391
Asp Met Thr Gly Ile Pro Thr Leu Asp Asn Leu Gln Lys Gly Val Gln

ttt gct ctc aag tac cag tcg ctg ggc cag tgt gtt tac gtg cat tgt    439
Phe Ala Leu Lys Tyr Gln Ser Leu Gly Gln Cys Val Tyr Val His Cys

aag gct ggg cgc tcc agg agt gcc act atg gtg gca gca tac ctg att    487
Lys Ala Gly Arg Ser Arg Ser Ala Thr Met Val Ala Ala Tyr Leu Ile

cag gtg cac aaa tgg agt cca gag gag gct gta aga gcc atc gcc aag    535
Gln Val His Lys Trp Ser Pro Glu Glu Ala Val Arg Ala Ile Ala Lys

atc cgg tca tac atc cac atc agg cct ggc cag ctg gat gtt ctt aaa    583
Ile Arg Ser Tyr Ile His Ile Arg Pro Gly Gln Leu Asp Val Leu Lys

gag ttc cac aag cag att act gca cgg gca aca aag gat ggg act ttt    631
Glu Phe His Lys Gln Ile Thr Ala Arg Ala Thr Lys Asp Gly Thr Phe

                          END SEQ ID NO: 3
gtc att tca aag aca tga tgtatgg ggattagaaa gaactcaaga cactcctgct    686
Val Ile Ser Lys Thr END SEQ ID NO: 2

tgatacagaa caaaaagagc ttaacaggac caacagggct taagcccaga cttgacgtaa  746

cagaaatgtg ccaataggta ataggtaatt tttctttctc tgacttgttt tgttttcttg  806

aaataacact gttgtgtggc tagaaaaaaa aaa END SEQ ID NO: 1              839
```

FIGURE 1

Figure 2

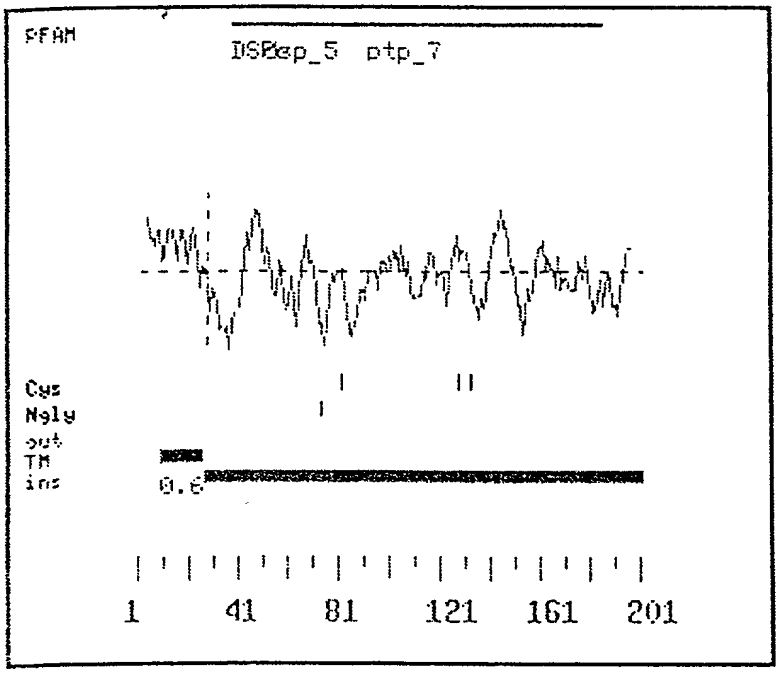


Figure 3

```
Alignments of top-scoring domains:
DSPc: domain 1 of 1, from 37 to 185: score 20.3, E = 3e-05
                   *->gpseIlphlYLGsystaseanlallkklgIthviNvteevpnpfeld
                         +I p + LG+ +  s     l +  ++  vi + ee + + +l+
        8843    37    WYHRIDPTVLLGALPLRS-LTRQLVQDENVRGVITMNEEYE-TRFLC 81

                   kkndrhytnayisknsgftylqiPnvdDhIYyhiawnhetki.skyfdea
                               ++ ++ ++l++   +         ++++  + + +d++
        8843    82 ------------NSSQEWKRLGVEQLRL--------STVDMTgIPTLDNL 111

                   vdFIdda...rqkggkVLVHCqAGiSRSAtliiAYLMktrnlslneAydf
                    + ++ a + ++ g  V+VHC+AG SRSAt+++AYL++ +++s +eA ++
        8843   112 QKGVQFAlkyQSLGQCVYVHCKAGRSRSATMVAAYLIQVHKWSPEEAVRA 161

                   vyvYhikerRcpiisPNfgFlrQLieyerk<-*
                   +       +R + i   +g+l  L+e+ ++
        8843   162 I-----AKIR-SYIHIRPGQLDVLKEFHKQ    185

//
```

Figure 4

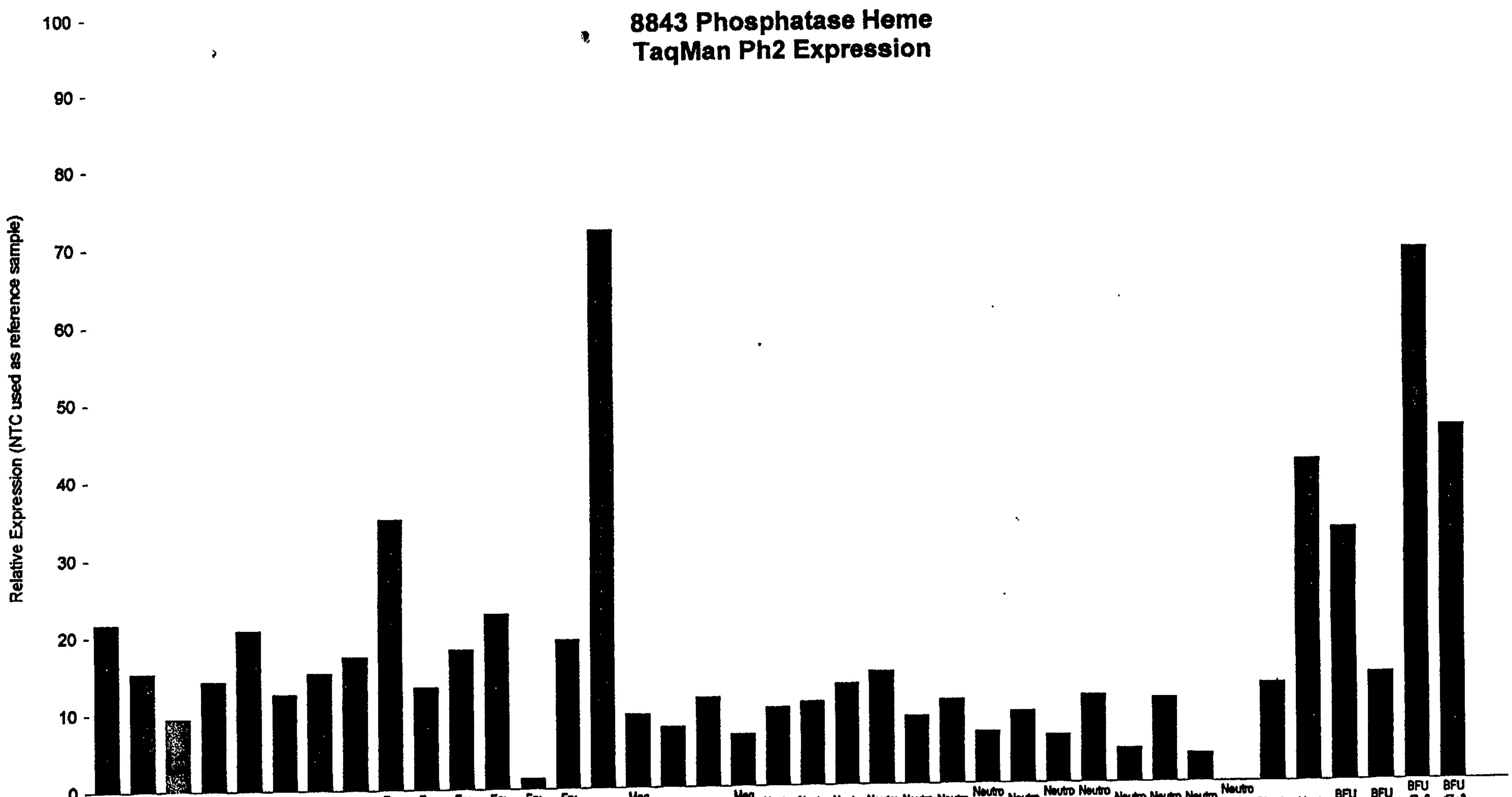
```
Alignments of top-scoring domains:
dsp_5: domain 1 of 1, from 50 to 185: score 18.3, E = 5.6e-05
                   *->gpseilph.lYLGsysdaseanlallkklgIthviNvteevpnnfel
                       p + l ++l +  + + +     +++++    +  N +
        8843    50    LPLRSLTRqLVQDENVRGV---ITMNEEYETRFLCNSS--------- 84

                   kkkndryytneyiskgsgftylqiPnvdDIyyhiawntetki.skyleea
                                   ++  +l++  +         +t++  + + l+++
        8843    85 ----------------QEWKRLGVEQLRL-------STVDMTgIPTLDNL 111

                   vefIeda...ekkggkVLVHCqAGvSRSAtlviAYLMktrnlslrdAydf
                    + ++ a + ++ g  V+VHC+AG SRSAt+v+AYL++ +++s ++A ++
        8843   112 QKGVQFAlkyQSLGQCVYVHCKAGRSRSATMVAAYLIQVHKWSPEEAVRA 161

                   vyvYhikerRcpiisPNfgFlrQLieyerk<-*
                   +      ++R + i    g+l  L+e+ ++
        8843   162 I-----AKIR-SYIHIRPGQLDVLKEFHKQ    185
```

8843 Phosphatase Heme
TaqMan Ph2 Expression



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Beta	19	19	19	18	19	19	19	19	19	21
Rel exp	22	15	9	14	21	12	15	17	35	13

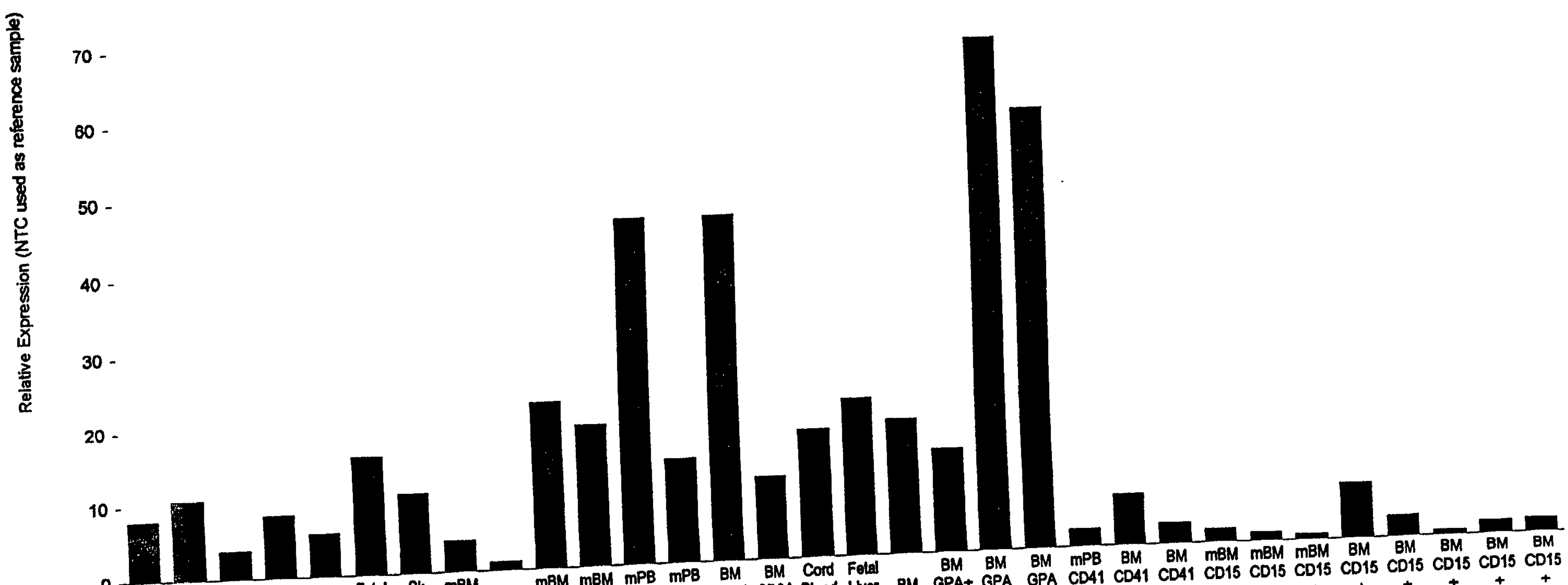
	Ery d12 LF23-8	Ery d12 LF24-10	Ery d14 GPA+ LP31-	Ery d14 CD38 +	Meg 48hr LF790	Meg d7 LP31-2	Meg d12 LF102	Meg d12 LF35	Meg d14 LP31-5	Neutro phil d4 LF71
8843	26	26	28	25	29	25	25	25	24	24
Beta	19	19	18	19	25	18	18	19	18	17
Rel exp	18	23	1	19	72	10	8	12	7	10

	Neutro phil d4 LF78	Neutro phil d6 LF28	Neutro phil d6 LP27	Neutro phil d6 LP71	Neutro phil d6 LP78	Neutro phil d7 LP41-3	Neutro phil d8 LF78	Neutro phil d11 LF78	Neutro phil d12 LP28B	Neutro ph d13 LF78
8843	24	24	26	24	24	26	24	24	23	24
Beta	18	18	19	17	17	18	17	17	17	17
Rel exp	11	13	16	9	11	7	10	8	12	5

	Neutro ph d14 LF71	Neutro ph d14 LF78	Neutro ph d14 LP31-8	Platelets	Mast cells	BFU d7 LP79	BFU d7 LP95	BFU d7+3 Epo LP81	BFU d7+3 Epo LP104	NTC
8843	24	25	34	28	24	24	23	24	28	40
Beta	17	17	18	19	20	19	17	20	24	40
Rel exp	11	4	0	13	42	33	14	69	48	

Figure 5

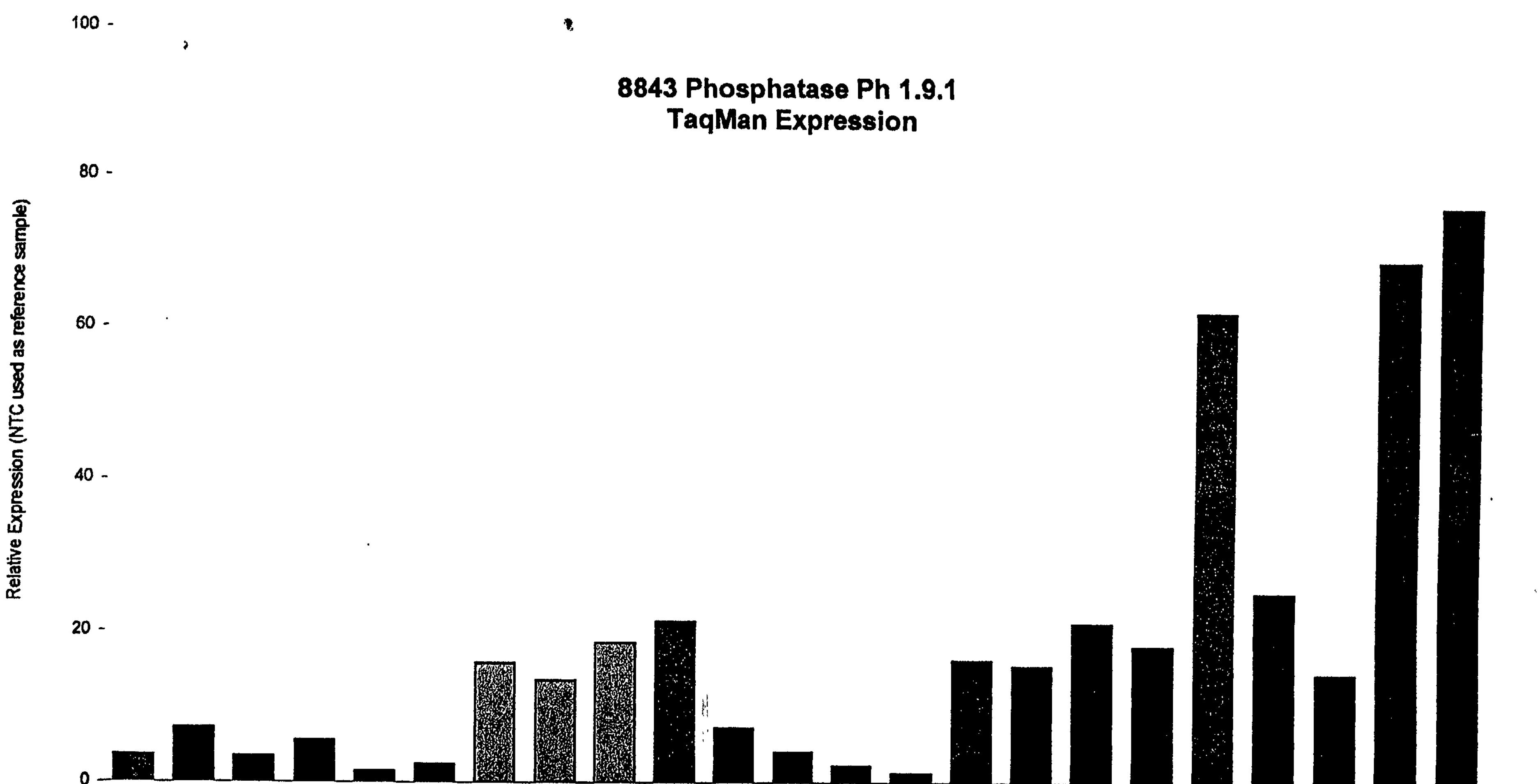
8843 Phosphatase Heme
TaqMan PH2 Expression



	Lung	Colon MPI 80	Splee n MPI 380	Kidne y MPI 58	Liver NDR 200	Fetal Liver MPI 425	Sk Muscl e MPI 167	mBM MNC LP13 9	mBM MNC LP7	mBM CD34 + LP92	mBM CD34 +LP1 43	mPB CD34 + LP57	mPB CD34 + LP70	BM CD34 + LF68	BM CD34 + LF81	Cord Blood CD34 +	Fetal Liver CD34 +	BM GPA+	BM GPA+ LP34- 1	BM GPA Lo LP69	BM GPA Lo LP82	mPB CD41 + CD14-	BM CD41 + CD14-	BM CD41 + CD14-	mBM CD15 + LP15	mBM CD15 + CD11	mBM CD15 + CD11	BM CD15 + CD11	BM CD15 + CD11	BM CD15 + CD11	BM CD15 + CD34-	BM CD15 + CD34-
8843	24	26	24	26	28	26	27	25	26	23	24	28	25	24	24	24	25	27	26	23	25	26	27	26	26	27	27	25	25	26	27	27
Beta	17	19	16	19	20	20	20	17	16	18	18	24	19	20	17	19	20	21	20	19	21	19	20	18	16	17	17	18	16	15	18	18
■ Rel exp	8	11	4	8	6	16	11	4	1	22	19	46	14	46	11	17	21	18	14	68	59	2	7	3	2	1	1	8	3	1	2	2

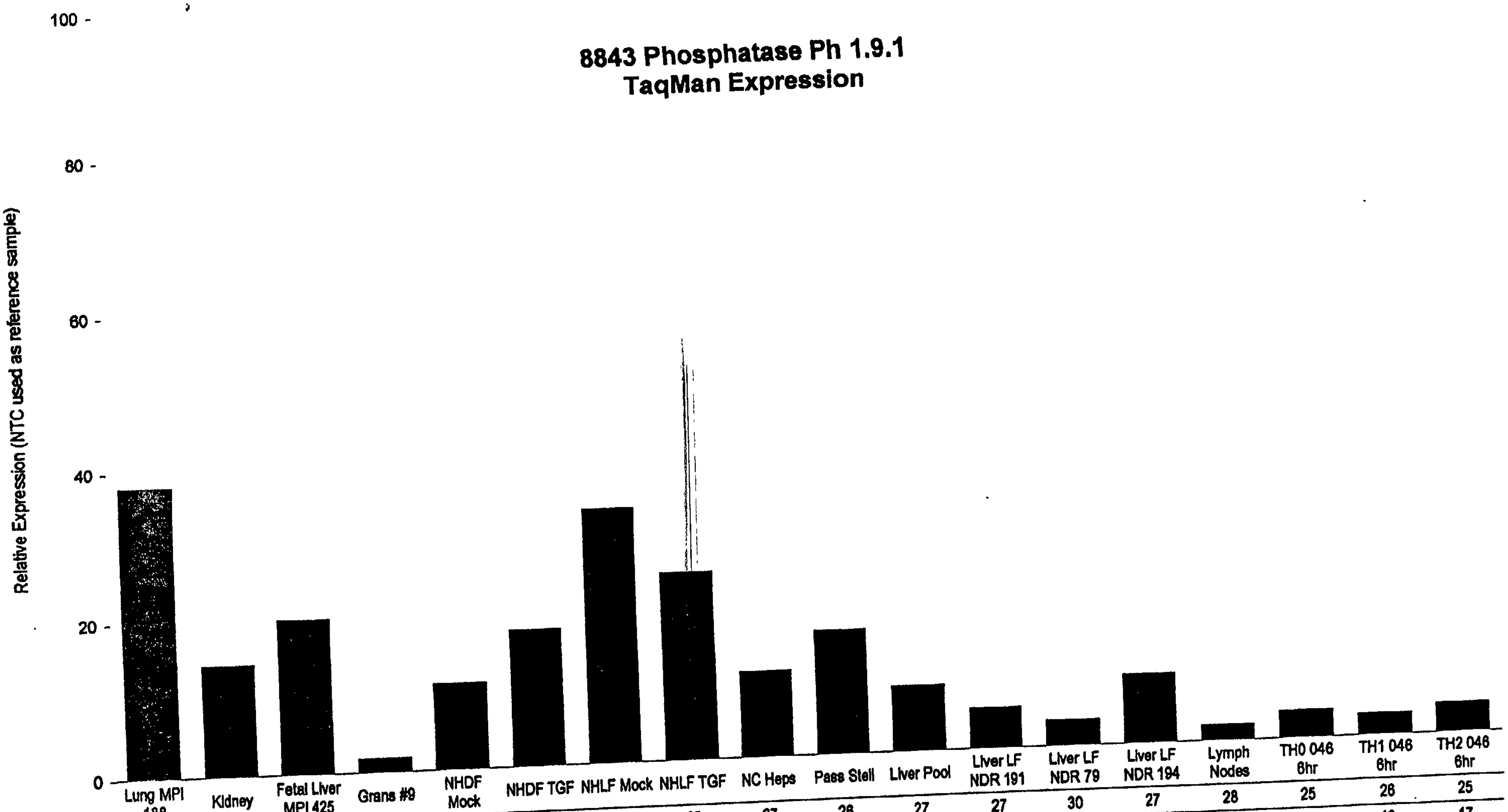
Figure 6

8843 Phosphatase Ph 1.9.1
TaqMan Expression



	CD8	CD14	PBMC Resting	CD19	CD3	mBM MNC LP139	mPB CD34+ LP70	BM CD34+ LF68	Cord Blood CD34+ LP121	Erythroid	Meg	Neut d14	BM CD15+ CD14- LP32	mBM CD15+ CD11b-	BM GPA+	VZV Mock	VZV 18hr	VZV 72hr	K562	HL60	Molt4	Hep3b Norm	Hep3b Hyp	NTC
8843	27	23	30	26	26	26	27	25	26	26	26	25	27	29	28	24	25	26	25	24	25	24	24	40
Beta	19	16	22	18	17	18	21	19	20	20	19	17	18	19	22	18	19	20	21	19	19	20	20	40
■ Rel exp	3	7	3	5	1	2	16	14	19	21	7	4	2	1	16	16	21	18	63	26	15	69	76	

Figure 7



	Lung MPI 188	Kidney	Fetal Liver MPI 425	Grans #9	NHDF Mock	NHDF TGF	NHLF Mock	NHLF TGF	NC Heps	Pass Stell	Liver Pool	Liver LF NDR 191	Liver LF NDR 79	Liver LF NDR 194	Lymph Nodes	TH0 046 6hr	TH1 046 6hr	TH2 046 6hr
8843	27	28	27	31	25	24	24	25	27	28	27	27	30	27	28	25	28	25
Beta	22	22	21	21	19	18	19	20	20	20	20	20	21	20	19	17	18	17
■ Rel exp	38	14	20	2	11	18	33	25	11	16	8	5	3	9	2	3	3	4

Figure 8

Attorney's Docket No.: 10448-088001
Client's Ref. No.: MPI2000-345; 100-645590

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled 8843, A NOVEL HUMAN DUAL SPECIFICITY PHOSPHATASE FAMILY MEMBER AND USES THEREOF, the specification of which:

[X] is attached hereto.
[] was filed on _ as Application Serial No. _ and was amended on ________________.
[] was described and claimed in PCT International Application No. ________________ filed on ________________ and as amended under PCT Article 19 on ________________.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Louis Myers, Reg. No.: 35,965
Timothy A. French, Reg. No.: 30,175
Diana M. Collazo, Reg. No.: 46,635
Laurie Butler Lawrence, Reg. No.: 46,593

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Address all correspondence to LOUIS MYERS at:

FISH & RICHARDSON P.C.
225 Franklin Street
Boston, MA 02110-2804

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Full Name of Inventor: NADINE WEICH

Inventor's Signature: ________________________________ Date: ________________
Residence Address: 70 Park Street #53, Brookline, MA 02446
Citizenship: United States of America
Post Office Address: 70 Park Street #53, Brookline, MA 02446

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Ala Leu Pro Leu Arg Ser Leu Thr Arg Gln Leu Val Gln Asp Glu Asn
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Cys Asn Ser Ser Gln Glu Trp Lys Arg Leu Gly Val Glu Gln Leu Arg
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